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CYTOLOGICAL AND OTHER FEATURES OF VARIANT PLANTS PRODUCED FROM X-RAYED SEX CELLS OF NICOTIANA TABACUM

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(WITH ELEVEN FIGURES)

As already reported (3), large numbers of variant individuals occur in selfed progeny from X-rayed sex cells of *Nicotiana tabacum* var. *purpurea*. In the present report a more detailed description of the external morphology of certain variant types and particularly of their cytological condition is included. An analysis of second and third generation progenies of variants is being made and will be the subject of a subsequent report. Plants from sex cells exposed to radium emanations and from seeds and seedlings subjected to ultra-violet, radium, and X-rays are being grown.

NATURE AND IMMEDIATE EFFECTS OF TREATMENT

During January, and again during October, 1927, pot-grown plants of *N. tabacum* var. *purpurea* (U.C.B.G. 014), comparable in size with the one shown in fig. 1*a*, were taken from the greenhouse and placed under a Coolidge tube housed in a fan-cooled lead room. Previous to exposure all open flowers and seed capsules were removed, leaving buds of all sizes, from those extremely minute to those less than 12 hours from anthesis. Distance from the target in the case of individual buds was subject to some variation, despite

efforts by trimming and tying to orient all buds in one plane. The average distance was 30 cm. At the January X-raying one plant was irradiated for 10 minutes and another for 20 minutes, while during the October X-raying two plants were exposed each for 15 minutes. On a number of occasions during the past year plants of other *Nicotiana* species have been irradiated under identical conditions, except that time and target distance have been varied.

In every case a standard, unshielded, deep therapy tube was employed at an effective potential of 50 kv. The current through the tube was 2.8 milliamperes.

In the case of both plants of the January X-raying, most of the smaller flower buds were abscissed within 48 hours. Immediately after treatment aceto-carmine smears of anthers from buds of various sizes were taken. Practically all the buds containing P.M.C. stages were abscissed, whereas all minute buds up to 4 mm. over-all were retained along with all large buds containing maturing pollen and E.M.C. in all stages. All these buds matured normal flowers and in every case set seed on self pollination. Without going into the evidence in detail, it should be noted that incidence of bud abscission at such stages is not a criterion of effective dosage. The data in hand indicate that the relation of irradiation to the abscission reaction and factors controlling or modifying it should provide an interesting field for carefully controlled investigation.

The four plants involved in the two X-rayings showed no visible somatic modification, and there was no abscission of later and adventitious buds on the terminal inflorescence or on the inflorescences subsequently produced on laterals. One plant of the October X-raying exhibited almost at once an acceleration of growth, and at times treated plants of other *Nicotiana* species have given striking evidence of the same phenomenon. Statements in the literature as to the relation of growth to irradiation are often contradictory, and again the interest of careful investigation of this problem suggests itself. *N. rustica* might prove to be useful material in such case, since on three different occasions pot-grown plants of variety *pumila* irradiated just previous to first flowering have shown an immediate and very marked growth acceleration.

The statements just made as to the absence of any visible somatic

modification following irradiation hold for all treated species except *N. langsdorffii*. In this case all buds of the terminal inflorescence were abscissed immediately after treatment and all the first flowers on laterals were abnormal. After a short time only normal flowers were produced, however, and both abnormal and normal flowers set full capsules of seed. A possibly comparable "recovery" has been referred to in the literature dealing with both the physiological and genetic effects of irradiation.

TABLE I

OCCURRENCE OF VARIANTS IN TEN POPULATIONS FROM SELFED SEED PRODUCED BY X-RAYED SEX CELLS OF NICOTIANA TABACUM VAR. PURPUREA (U.C.B.G.014)

No.	Culture no.	Exposure (minutes)	Condition of bud	No. variant	No. normal	Total	Percentage variant (approximate)
1.....	27151	10	♀ prophase (?)	26	169	195	
2.....	155	10	♀ prophase (?)	10	188	198	
3.....	149	10	24 hours before anthesis	4	28	32	7
4.....	153	10	Anthesis	7	183	190	
5.....	150	20	♀ I-M (?)	33	152	185	
6.....	154	20	♀ I-M (?)	136	32	168	
7.....	152	20	24 hours before anthesis	12	61	73	50
8.....	G27101*	15	♀ I-M (?)	10	63	73	
9.....	110*	15	♀ I-M (?)	3	33	36	11
10.....	112*	15	♀ prophase (?)	4	35	39	
Totals.....				245	944	1189	20

*These three progenies were from seed following the October X-raying and flowered in the greenhouse from December to February. The conditions under which they grew made it difficult to estimate the expression of plant characters, particularly vegetative ones. They are included in tables I and II merely to indicate that the second X-raying of *purpurea* gave rise to variants of the same type as those which occurred in the previous experiment.

OCCURRENCE AND NATURE OF VARIANTS

During the summer of 1927, 1040 plants from selfed seed of the January X-raying were grown, in seven populations. In the greenhouse during the past winter three populations from selfed seed of the October X-raying were grown (table I, nos. 8, 9, 10). In both cases plants differing in practically all characters from the control occurred and are referred to as "variants." Table I shows the occurrence of variant plants in the various populations. For assistance

in classification of the field populations I am indebted to Miss PRISCILLA AVERY.

In the greenhouse a population (G27102) from control 014 ♀-X-rayed ♂ was grown. The pollen matured from a bud in which

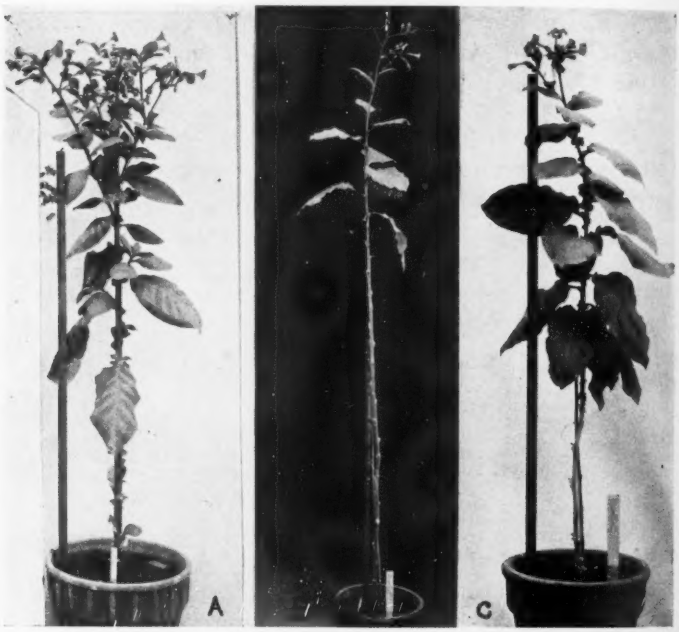


FIG. 1.—*a*, 27014, *Nicotiana tabacum* var. *purpurea*, approximately equivalent in condition to plants which were irradiated; *b*, *c*, G27101P71 and 110P5, variants from a repeat X-raying of 014 (table II, nos. 17 and 18).

the pollen was, apparently, almost mature when X-rayed. Six quite striking variants appeared in a population of forty-four plants. This result suggests that irradiation of mature pollen may effect genetic modification, a point which should prove of some importance.

Often variant characteristics were clearly shown in early seedling stages, and usually the mature plants from variant seedlings were also strikingly variant in external morphology. For example, in 27154 (table I, 6), of eighteen abnormal seedlings held in pots in the

FIG. 2.—Plants from X-rayed sex cells: *a*, 27154P004, extreme variant type containing fragmented chromosomes (figs. 8, 9, 10); *b*, 27150P003, equivalent to control and showing 24_{II} at I-M.

greenhouse, sixteen gave variants at maturity. Two, on the other hand, gave apparently normal plants, and in a number of instances it has been observed that variant seedlings of abnormally slow growth produced vigorous basal laterals from which a mature plant, normal in external appearance, was built. In general, conspicuously slow early growth in the case of young plants of normal appearance

FIG. 3.—Plants from X-rayed sex cells: *a*, 27154P014; *b*, 27150P001 (table II, nos. 6 and 2).

seems to be a fairly reliable index of variant character at maturity. In rare instances very rapid maturity has occurred and has usually been correlated with variation.

An attempt is made by figs. 1–6 and by the data included in table II to give some picture of the extent of variation in all plant characters exhibited by the many variants produced from selfed seed of flowers which matured from X-rayed buds.

It is obviously difficult to express many of the data included in table II in any but relative terms. It should be clear, however, that in the plants listed the distinction from the control in character ex-

pression was sharp, and in the majority of cases involved most of the vegetative and floral characters. In addition to the characters listed, many variations in leaf number, leaf shape, venation, position of the floral organs, lobing of the stigma, number of carpels, and so forth, were noted. The fertility data in table II are of relative significance and are based upon field observations of capsule development

FIG. 4.—Variation in leaf shape: 014 (control) at bottom; 27154P80 (table II, no. 10) above, left; 27154P177 (table II, no. 15) above, right.

and contents. The results of crosses to the control and of germination studies of variants and normals indicate that, as might be anticipated, various degrees of gametic and zygotic lethality are involved.

The results of irradiation in these experiments have involved effects, presumably largely nuclear, which express themselves upon the immediate progeny. In the case of plants apparently no such effects, comparable in extent and character of resulting variation, have been hitherto reported. The figures given in table I indicate that it may be possible to place the relation of X-ray dosage for sex

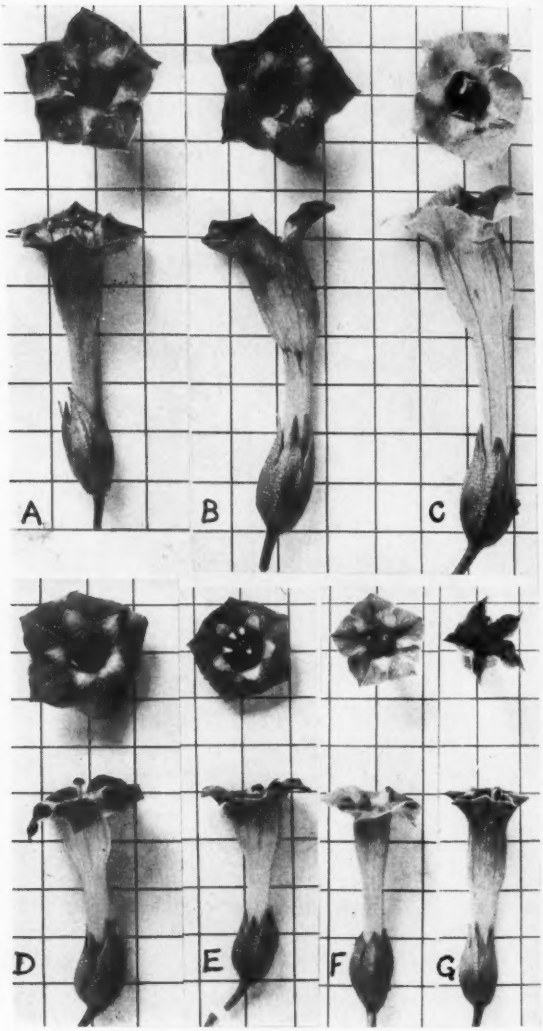


FIG. 5.—Variation in flower form: *a*, 014 (control); *b*, 27154P168, flower larger than 014 and color darker; *c*, 27154P23, larger and color lighter; *d*, G27110P5, flower smaller and stigma exserted; *e*, G27101P46, smaller and color darker; *f*, 27154P158, smaller and color lighter; *g*, 27154P129, smaller, "limb erect" type.

cells to incidence of variation in immediate offspring, on at least a semiquantitative basis. The increase in total number of variants with increase in length of exposure would appear to have some defi-

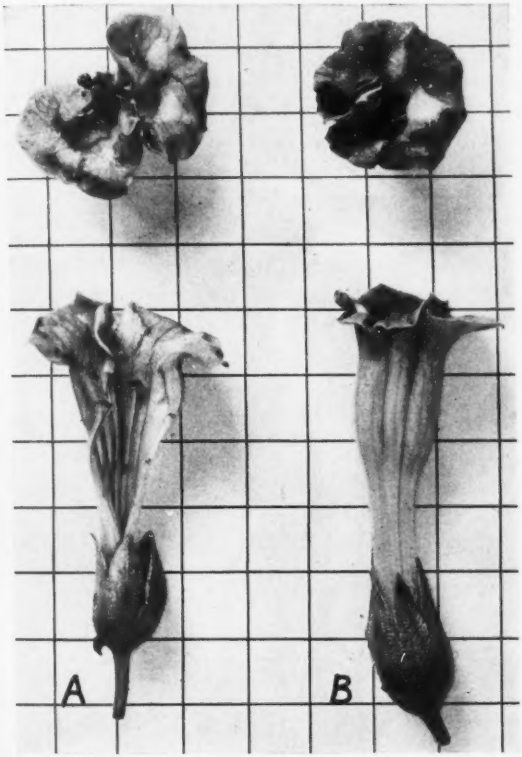


FIG. 6.—Extreme variation in flower form: *a*, 27151P200, "split" type; *b*, 27154P142, "pleated" type.

nite significance. A similar direct relation appears to exist where the condition of the sex cells during treatment was the same. Contrasting the occurrence of variants from buds in which the pollen and embryo sacs were mature (table I, 3, 4, 7), the 10-minute exposure gave approximately 5 per cent whereas the 20-minute exposure gave approximately 15 per cent of variants. A similar contrast in the case

TABLE II

No.	Culture no.	Figure no.	Flower size (mm.) length: spread	Flower color	Flower form	Habit	Leaf	Fertility (percentage)	Cytology at I-M	Remarks
1.....	27014* (control)	1a, 4, 5a, 7a, 11b	50:36	Red	Normal	Medium	Sessile	100	24II	.....
2.....	27150P001	3b	42:34	Red	Slender and fluted	Dwarf	Sessile	0–25	.....	Leaves thick, gray green
3.....	P003	2b	50:32	Red	Normal	Medium	Sessile	100	24II	Equivalent to control
4.....	27154P002	10b, 11c	39:24	Red dark	Incised limb	Dwarf	Sessile	0	23II+1f	Leaves gray green, linear
5.....	P004	2a, 8a, 9, 10c	43:33	Light red	Extra lobes	Dwarf	Sessile	0–50	23II+2f(?)	Leaves thick, stigma and anthers low
6.....	P014	3a	59:28	Light red	Tube slender	Medium	Petiolate	0	24II	Leaves lanceolate
7.....	P23	5c	67:38	Pink	Normal	Medium	Petiolate	0–75	24II+1I or 23II+2I	Very large flower
8.....	P24	8b	36:25	Red	Tube slender	Dwarf	Sessile	0	24II+1f	.....
9.....	P36	.....	36:26	Red	Tube fluted	Dwarf	Sessile	0–25	23II+1I(?)	Thick, linear leaves
10.....	P80	4	53:29	Red	Normal	Medium	Petiolate	75	24II	.....
11.....	P129	5g	40:25	Red	Limb erect	Medium	Sessile	25	.....	.....
12.....	P158	5f	37:22	Light red	Normal	Short	Sessile	75	.....	Small leaves and flowers
13.....	P162	7b, c	55:31	Lively red	Limb erect	Short	Sessile	75	23II+1I	Large leaves
14.....	P168	5b	57:41	Dark, lively red	Normal	Dwarf	Petiolate	25–50	22II+2f(?)	Stigma low
15.....	P177	4	47:35	Light red	Broad	Medium	Petiolate	0	Variable	Venation irregular and weak
16.....	G27101P46	5e	35:24	Dark, lively red	Limb fluted	Medium	Sessile	75	.....	Internodes short, auricles large
17.....	P71	1b	58:35	Light red	Calyx reduced	Tall	Sessile	75	.....	Very tall, fast growing
18.....	G27110P5	1c, 5d	45:30	Light red	Limb erect	Dwarf	Petiolate	25–50	.....	Stigma exserted

* For *N. tabacum* var. *purpurea* (014) the details of habit, leaf shape, and flower form may be obtained from figs. 1a, 4, and 5a.

of immature pollen and E.M.C. in division stages (table I, 1, 2, 5, 6) shows approximately 10 per cent for the shorter and 45 per cent for the longer exposure. As might be anticipated, the percentage of variants from buds where one set of sex cells was in meiotic stages was higher than from buds in which both sets were practically mature.

These data as to the relation of exposure time and stage of sex cell development to the production of variation are obviously too fragmentary to permit the drawing of larger conclusions. On the other hand, it is doubtful to what extent it will be possible in the future to supplement them in a significant fashion in the case of the material used. One major difficulty would seem to be the variations in the stages of maturity of sex cells in a given bud. A small ovary of *tabacum* contains from 1000 to 1500 E.M.C. in all stages, from early prophase to second telophase. In the case of P.M.C. the series is not so extensive, but it is apparently readily extended by changes in temperature, and perhaps by other factors also. From this point of view alone, since the character, extent, and significance of genetic modification following irradiation would appear to be intimately related to the stage of maturity of the given sex cell, the problem of relation of dosage to incidence of a particular category of variation is complicated. In this connection, the possible use of mature pollen should be considered, particularly in the light of the results just noted.

Cytological features exhibited by variants

Some forty plants of 27150, 154, and 155 (tables I, II) were studied cytologically. In the majority of cases meiotic stages were examined in detail, while in a few only pollen tetrad counts were made. In most cases both aceto-carmine preparations and paraffin sections were studied. Some examination of E.M.C. and of somatic mitosis was made. The paraffin material was fixed in modifications of Karpechenko's formalin-chrom-acetic solution and stained with iron haematoxylin. The figures illustrating cytological conditions were drawn from such preparations unless otherwise noted. Miss Mabel L. Ruttle has given valuable assistance in connection with these cytological investigations.

The complications involved in analysis (both genetic and cytological) when dealing with selfed progenies of eggs and sperms, both of which may possess nuclear modification as a result of irradiation, are obvious. As noted, it was difficult to arrange the variants into classes on the basis of equivalence in external morphology, either total or partial, and it may be that the following classification of the cytological behavior exhibited by certain of these variants has only relative significance. The designations of classes which follow are descriptive of the conditions observed during mitosis or meiosis. Certain of these cytological phenomena in irradiated material have been described by KOERNICKE (**5, 6**), STEIN (**9**), PEKAREK (**8**), and others (cf. HERTWIG **4**).

CHROMOSOME NUMBER AND BEHAVIOR NORMAL

Of twenty-nine plants on which cytological data are most extensive, two were entirely normal in external morphology and fully fertile (fig. 2*b*). They were likewise indistinguishable from the control cytologically, and exhibited 24_{II} at I–M in P.M.C. and E.M.C. The diaphase and metaphase showed close pairing, and there was no evidence of lagging at I, II, or in microcyte number at the pollen tetrad stage. Chromosome conditions of this sort are referred to as "normal." Seven other plants, all variants, were found to be normal cytologically (figs. 3*a*, 4; and table II, 6, 10). Their fertility was much reduced in crosses with the control, and one of these seven plants was completely sterile. In this first class, then, involving normal cytological conditions, wide variations in character expression and fertility occurred. At one extreme were plants equivalent to the control, and at the other individuals differing from it in all plant characters and more or less completely sterile.

NON-CONJUNCTION

Five plants were included under this heading because, although they sometimes gave counts of 24_{II} at early I–M, they showed loose pairing at diaphase, chromosomes off the equatorial plate at I and II–M, and large numbers of microcytes at the tetrad stage. Since unpaired chromosomes appeared during I, these cases might be included under the next category, that is, "unpaired chromosomes."

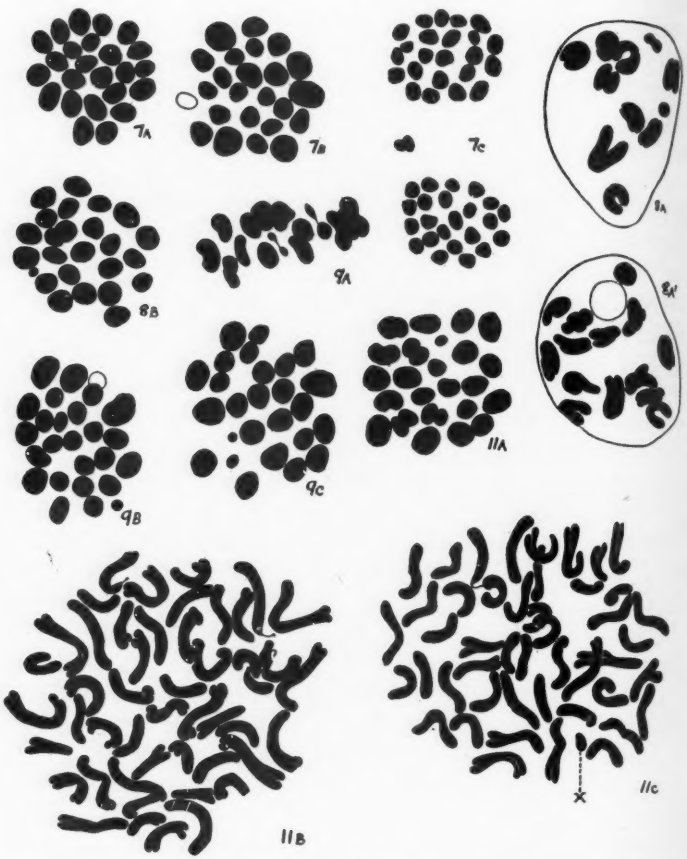
On the other hand, in those instances next described the I–M count was always relatively clear, while here the variation in the number of bivalents and univalents from one M.P.C. or E.M.C. to the next was so great that no final decision could be made as to the extent of conjugation.

The fertility of this group was relatively very low, only two plants giving any seed. In external morphology they were highly abnormal, with the exception of one plant which was a replica of the control but exhibited a greatly reduced fertility. In this plant 24_{II} could at times be counted at I–M; but there were always lagging chromosomes at interkinesis, and at II–M there were usually less than 24 in single plates with from two to five chromosomes in the plasma, and over 40 per cent of microcytes. One striking variant consistently showed from one to three chromosomes off the plate at I-M, and at diaphase 24 units, some very loosely associated. There were over 30 per cent of microcytes and the plant was sterile. Finally there was a highly variant, sterile plant (fig. 4) which apparently possessed a normal somatic chromosome complement, but exhibited a highly variable condition at I–M, the chromosomes being scattered as bivalents and univalents over the spindle and later distributed very irregularly.

Unpaired chromosomes

The chromosome situation in eight plants suggested that they might be the products of relatively simple meiotic aberration following irradiation. Four showed 23_{II} and 1_{I}, and three showed 24_{II} and 1_{I} at I–M in P.M.C.; the eighth plant possessed either 22_{II} and 1_{I} or 23_{II}.

In fig. 7*b* and *c*, typical I and II–M conditions in 27154P162 are illustrated, together with I–M of the control (*a*). In chromosome behavior, 27154P162 corresponded to monosomic *tabacum* individuals, which previously had occurred in our cultures (**2**). There were clearly 23 bivalents, and a somewhat smaller, paler chromosome which was, in many cases, off the equatorial plate at early I–M (fig. 7*b*) and lagged at I–T. In one case at II-M (fig. 7*c*) 23 chromosomes could be counted in both plates and there was a chromosome dividing in the plasma. In other P.M.C. 23 and 24 were counted at II-M. Approximately 15 per cent of microcytes occurred. Corresponding



FIGS. 7–9, 11.—Fig. 7, I and II-M of P.M.C.: *a*, 27014 (control), I-M 24_{II}; *b*, 27154P162 (table II, no. 13) I-M 23_{II} and 1_{I} (in outline); *c*, II-M, aceto-carmine preparation, 23 chromosomes in each plate and one in the plasma (after partial division at I-A). Fig. 8, *a*, *a'*, 27154P004 (table II, no. 5) diaphase, P.M.C., 23_{II} plus two chromosome fragments; *b*, 27154P24 (table II, no. 8) I-M, P.M.C., 24_{II} plus one fragment. Fig. 9, 27154P004, P.M.C.: *a*, late I-M, side view, all chromosomes not shown, disjunction beginning, chromosome fragments undergoing early and unequal division; *b*, I-M, 24_{II} plus 1_{I} and a fragment; *c*, 24_{II} plus two fragments. Fig. 11, *a*, late I-M, E.M.C., 27154P002, 23_{II} plus one chromosome fragment; *b*, somatic metaphase of 014 (control); *c*, same, 27154P002, 47 chromosomes and one chromosome fragment (*x*).

chromosome behavior was found in the remaining three "monosomic" individuals.

In the case of the "trisomic" plants, there was at times some difficulty in deciding whether the chromosome garnitures were not 23_{II} plus 2_{I} rather than 24_{II} plus 1_{I}. The II-M showed in most cases 24 and 25, or 24 in each plate with a chromosome in the plasma. More than a single chromosome was at times seen in the plasma at interkinesis. A precocious division of the univalent at I-A, sometimes observed, may account for this variation. On the other hand it may appear after further study that certain of these variant individuals should be included in the previous category of chromosome behavior (non-conjunction). There was little evidence of trivalent occurrence at I-M which corresponds to the condition reported in the case of a known *tabacum* trisomic (1).

None of these eight plants, in most cases presumably the products of non-disjunction occurring subsequent to irradiation, was equivalent in external morphology to any monosomic or trisomic *tabacum* which has appeared in our cultures. In the case of two of the four "monosomic" variants, a strong resemblance to "fluted" *tabacum* (2) was noted. This was also true of a number of the variants in the different X-ray populations, and a certain proportion of the total number of plants of this type should, perhaps, be assigned to induced or spontaneous occurrences of "fluted." The majority, differing markedly from "fluted," undoubtedly involve genic modifications or chromosomal distinctions in addition to the loss of the chromosome which results in the typical change in external morphology.

It would appear, then, that following irradiation of *tabacum*, as in other material also, a considerable increase in the incidence of non-disjunction may be expected to occur, this more simple chromosomal modification being often accompanied by other changes.

Chromosome fragmentation

Five plants showed evidence of chromosome fragmentation at meiotic stages, and for one of them the presence of a small chromosome in the somatic complex has been demonstrated. Extended studies of chromosome behavior were made in three plants, 27154P002, P004, and P168. As will be noted in table II (4, 5, 14), all three

plants were highly variant in external morphology (figs. 2*a*, 5*b*). Two showed a reduced and variable fertility, while the third was

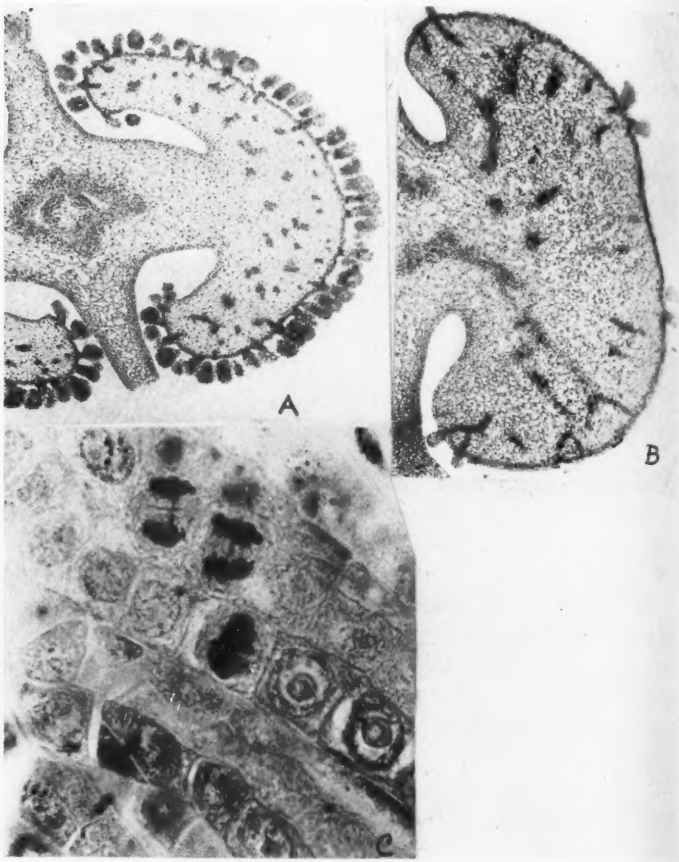


FIG. 10.—*a*, *b*, cross-sections of ovary at anthesis; *a*, 014 (control); *b*, 27154P002 (table II, no. 4) to show absence of ovules; *c*, 27154P004, longitudinal section of root tip to show lagging chromosomes at telophase.

completely sterile. Figs. 8–10 illustrate portions of the cytological evidence obtained.

The presence in these plants of minute chromosomes at diaphase

and I and II-M was clear. In P002 and P168 there were 22_{II} or 23_{II} plus one such minute unit, in P004 probably 23_{II} plus two fragments. Of the remaining two plants of this category, the first (P24, fig. 8*b*) possessed probably 24_{II} and one chromosome fragment, the second 23_{II} and one fragment. As the various figures show, the minute units in these five plants were similar in size and form. In meiotic stages some of them were at first seen as appendages attached by delicate strands to an otherwise normal bivalent, and were thus described in an earlier report (3). Imperfect fixation may have been responsible for the earlier decision as to their relation to other chromosomes, although at times and with otherwise successful technique they have again appeared as appendages, particularly at diaphase. At I-M their position either within or without the equatorial plate of bivalents is variable.

Neither at diaphase nor at I-M was there any evidence that the chromosome fragments existed in pairs. Usually they were distributed along with the bivalent partners, and at times divided at II-A (7). They may divide at I, however, and their "halves" complete passage to opposite poles. Such division is almost always precocious and almost complete before the disjunction of surrounding bivalents has been initiated. It also often appeared to be unequal (fig. 9*a*), so that elements of different size went to opposite poles.

In 27154P004 the diaphases usually showed close pairing (fig. 8*a*, a^1), although a number of P.M.C. were seen in which it was not so precise. There was also some variation in the number of bivalents and univalents at I-M. There were even early I-M in which not less than 45 units could be counted, and, correspondingly, cells at II-M with the products of division of such a "non-reductional" I-M. In such cases the chromosomes at I and II-M were smaller than normal bivalents or products of their disjunction. Such P.M.C., exhibiting an almost complete failure of conjunction, were rare, whereas such conditions as are shown in figs. 8 and 9 were not uncommon. Fig. 9*b* exhibits 26 units, of which 24 are bivalents, one a chromosome fragment and one a smaller, paler chromosome corresponding in appearance to the univalent in monosomic *tabacum*. It is probable that a second chromosome fragment was present but obscured because of close association with one of the larger bivalents. In fig. 9*c* there are

again 26 units, but in this case two chromosome fragments appear together with 24_{II}. In the diaphase shown in fig. 8*a*, *a*[1], there are 23_{II} plus two fragments. In other words, there was evidence of variation from one P.M.C. to another in the number of chromosomes present at diaphase and I-M, even after every effort was made under the microscope to obtain evidence which would provide grounds for interpreting these results on a uniform basis.

It was not possible to obtain somatic counts from P004. In root tips there was no evidence of degeneration of cell lineages but chromosome lagging was frequently observed. In fig. 10*c* an earlier and a later telophase are shown, with chromatin strung between the polar accumulations. In one case the cell plate alone is serving to divide this chromatin strand. In some similar cells it did not appear possible that quantitatively equivalent portions were being thus separated to the daughter nuclei. This plant showed variation in degree of fertility from flower to flower. In one bud all P.M.C. and E.M.C. were maturing normally to give well-filled capsules on crossing to the control; other buds contained only degenerating sex cells in various stages of maturation; and still others showed one or two anthers in this condition with the remaining anthers normal. It may be that these abnormalities in somatic mitosis, occurring either in meristematic or archesporial tissue, may be responsible for the observed variation in fertility.

That the chromosome fragments which were so conspicuous at meiotic stages in P004 would have been identified in somatic mitoses is indicated by the evidence from P002. Fig. 11*a* shows a late I-M from an E.M.C. of this plant with 23_{II} and one fragment, while fig. 11*c* illustrates a somatic plate from a root tip which may be compared with the control in fig. 11*b*. The presence of a strikingly smaller chromosome is clear. P002 was highly abnormal in external morphology and completely sterile, degeneration of all the pollen taking place at late tetrad and the placentae being partially or wholly devoid of ovules. This latter point is illustrated in fig. 10*b*, which may be compared with fig. 10*a* from the control. In P002 the pairing at I-M was more precise than in P004, but there was much lagging during both I and II.

The case of 27155P001 is outstanding among the plants cytologi-

cal examination of which showed chromosome fragments, in that it closely approximated the control in external morphology and was fertile. There were 24 units at I-M, of which one was minute and chromosome behavior was normal throughout. Evidence as to the cytological condition of the remaining plant in this group is not so extensive. The majority of counts gave 24_{II} and a fragment, but in other cases there seemed certainly to be 23_{II} and a fragment. This plant was highly variant in all characters, and sterile.

Summary

1. Appropriate X-ray dosage applied to flower buds of *Nicotiana tabacum* may be followed by the appearance of large numbers of variant individuals in the selfed progeny.

2. There was evidence that variation can be induced by the irradiation of mature pollen.

3. Apart from the abscission of all smaller flower buds, no visible somatic effect followed the X-ray dosage employed.

4. The X-ray variants exhibited numerous types of modification of all vegetative and floral organs and reduction in fertility to various degrees.

5. Cytologically the variants examined proved (1) to be normal in chromosome number and behavior, (2) to show non-conjunction of one or more pairs of chromosomes, (3) to be the products of non-disjunctional phenomena, and (4) to possess fragmented chromosomes.

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A SPECTROPHOTOMETRIC STUDY OF REFLECTION OF LIGHT FROM LEAF SURFACES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 391

CHARLES A. SHULL

(WITH TEN FIGURES)

Introduction

The energy relations of leaves have received too little attention from plant physiologists during the last 25 years. The classical studies of BROWN and ESCOMBE (1) on the interchange of energy between the leaf and its surroundings furnish a very important source of information as to the quantitative income and outgo of energy during the processes of photosynthesis, transpiration, and thermal emissivity. They measured the solar radiation falling upon the leaf with great care, using an excellent type of radiometer for the determinations. By means of the same instrument they evaluated the coefficient of absorption of light by the leaf. The amount of energy utilized by the leaf for internal work, photosynthesis, and evaporation of water was carefully estimated from the actual use of CO_2 and the water vapor given off in transpiration; and the gain and loss of energy during periods of positive and negative thermal emission were calculated from data as to the rate of thermal change between environment and leaf with one degree of difference in temperature between them.

From these various measurements they proceeded to construct a balance sheet of energy income and outgo, which would account for 100 per cent of gain and loss of energy. Although they succeeded in working out such "revenue and expenditure" accounts for the leaf, and balanced them perfectly as good bookkeepers are supposed to do, it must be obvious from careful study of their work that these figures are not as accurate as they appear. One cannot help feeling that the data have been chosen carefully to make a perfect balance. The following example for *Helianthus annuus* gives an idea of these balance sheets, and is adapted from BROWN and ESCOMBE's paper:

ENERGY INCOME	CALORIES PER SQ. CM. PER MINUTE
1. By solar radiation	0.2746
2. By negative thermal emissivity	0.1817
Total income per sq. cm. per minute	0.4563
ENERGY OUTGO	
1. For photosynthesis	0.0033
2. For evaporation of water	0.3668
Total energy used in internal work	0.3701
Incident energy transmitted	0.0862
Total outgo per sq. cm. per minute	0.4563

In another instance the energy changes were calculated on the percentage basis, considering that the total energy receipt is 100 per cent. The disposal of energy received during insolation was accounted for by assigning to each energy-using process its percentage of the total. An example follows:

ENERGY UTILIZATION	PERCENTAGE
For photosynthesis	0.66
For transpiration	48.39
Total internal work	49.05
Transmitted energy	31.40
Positive thermal emissivity	19.55
Total energy used	100.00

One must admire the ingenious methods used by BROWN and ESCOMBE in obtaining the results presented. Their work represents possibly the best quantitative measurements of these physical processes of plant life in physiological literature, but the results as recorded are really too good to be accepted as free from choice of data such as would produce the balance sheet. Such perfect results can be obtained only when some of the determinations are made or approximated by "difference."

The most patent source of error in this work by BROWN and ESCOMBE is one of which they were cognizant, and to which they referred, but neglected because they thought it was a small error. This is the reflection of light from leaf surfaces. They pass over the matter of leaf reflection with the following statement:

The coefficient of absorption of leaf for solar radiation was determined by means of the same instrument (Callendar's self-recording radiometer) in the manner to be described in detail later on. It was taken as the difference between the solar radiant energy falling on the leaf in full sunshine and the amount transmitted, and takes no account of any possible reflection of radiation from the surface of the leaf. With perpendicular incidence the reflected radiation must be very small in amount, but it is well to bear in mind that, strictly speaking, the value of the coefficient of absorption employed includes this reflected portion.

The difficulty in this situation is that, even if we know the value of the reflection factor, and attempt to correct the coefficient of absorption, there is no simple way of correcting the figures in the balance sheet presented, to agree with the changed absorption quantity of energy. It seems therefore that the problems of energy utilization must be restudied, with the reflection factor taken into account.

The utilization of solar energy has recently been summarized diagrammatically from the BROWN and ESCOMBE data by SPOEHR (7), the disposal being accounted for in four fractions. Photosynthesis is represented as using 1 per cent of the total incident energy, while transpiration dissipates 50 per cent of it. About 30 per cent is transmitted, and the other 19 per cent is attributed to reradiation, which should include conduction of energy by the atmospheric gases, removal of energy by convection currents set up in the air from any cause, radiation proper, and light reflection from the surfaces of incidence. No attempt has been made to estimate quantitatively the part that each of these reradiation factors plays in the energy outgo from the leaf; no new data are presented covering any phase of the problem, so that SPOEHR leaves the problem just where BROWN and ESCOMBE left it in their contribution.

The interesting measurements of reflection of energy from leaf surfaces by COBLENTZ (2) were unknown to the writer when, some years ago, he called attention to this error in a brief discussion of transpiration as energy dispersal (6). In this paper it was assumed that the reflection from the leaf might be as much as 10 per cent, in view of the fact that a dead-black surface will reflect 1 per cent of the incident light. At the time this suggestion was made no data had come to the writer's attention upon which an accurate estimation of light reflection from leaves could be based, and the opportunity of making such measurements had not presented itself. In

this paper it will be shown that the estimate of 10 per cent was a very conservative figure.

It is fortunate that COBLENTZ included a few leaves of plants in his studies of the reflecting power of matte surfaces. In making his measurements he used a bolometer, a mirror spectrometer, and a fluorite prism. The angle of incident light in his work was 45°, the light coming from a Nernst glower, and the leaf was placed 6 cm. from the spectrometer slit. The earlier measurements made in 1908 extended from the visible into the infra-red as far as 3300 mμ, and in one case (*Liriodendron*) to 4400 mμ. The curves of reflectivity showed a decrease in reflection of energy with increase in wave length. In general the reflection in the infra-red region is small, but in the near infra-red it may be very considerable, 38 per cent at 950 mμ for instance.

In his later studies (1912) COBLENTZ measured the reflection of visible solar rays, mostly at 600 mμ, but in a few cases at 540 mμ, in the green region of the spectrum. Because it was felt that these data may not be as well known as they should be, permission was obtained to present them here. With some necessary modification, the data of COBLENTZ's table III and a part of table V are combined in table I.

Table I shows apparently that the general reflection from leaves at 45° incidence is not far from 25 per cent. This value is somewhat higher than is found for reflection normal to the surface. COBLENTZ points out the unexpectedly low results for *Verbascum*, considering its hairy surface. Measurements presented in this paper for the same plant are in complete agreement with COBLENTZ's work, in showing that hairs do not cause reflection of light to any marked extent. The two figures on transmission in table I show that about 20 per cent of the energy passes through the leaf under the conditions of the measurements.

The most recent study of light reflection is by POKROWSKI (4), who used a König-Martens spectrophotometer and measured the reflection from leaves of several species of trees at 90° incidence, comparing the reflection from that of MgO, the albedo of which is about 95 per cent. The species used were *Populus tremula*, *Tilia parvifolia*, *Fraxinus excelsior*, *Ulmus effusa*, *Castanea*, and *Acer*

platanoides. The leaves were tested at 1.5 months old, at various wave lengths from $\lambda = 450$ to 710 mμ. In the blue region of the spectrum he found 4–5 per cent of reflection, in the green 8–17 per cent, and in the red 4–10 per cent. These values are similar to those in the studies to be reported here for summer verdure. I have found much greater reflection in the case of spring verdure and autumn

TABLE I

REFLECTING POWER OF GREEN LEAVES, 45° INCIDENCE

PLANT	REMARKS	WAVE LENGTH λ MAXIMUM (PERCENTAGE)			
		540 mμ	600 mμ	950 mμ	4400 mμ
Trifolium pratense		23.9	21.3		
Syringa vulgaris	May 3	26.0	25.3		
	June 8		23.6		
	Transmission		19.6		
Robinia pseudacacia	May 3		25.4		
	June 8		23.9		
	Transmission		20.7		
Liriodendron tulipifera	Date?		21.9	38.0	5.6
	May 3	28.1	27.2		
	July 29		22.0		
	October 31	47.0	48.8		
Kalmia latifolia			23.2		
	Under side		27.7		
Tilia americana			26.9		
Ulmus rubra			25.7		
Quercus rubra	Young leaf		29.3		
	Dark green		21.8		
Verbascum thapsus			24.2		

coloration, in albino leaves, and in one case of extreme hairiness which gave a white surface (under surface of leaf of *Populus alba*).

POKROWSKI reports some transmission data, showing the transmission of 2–6 per cent at $\lambda = 480$ mμ, 10–29 per cent at 550 mμ, and 4–10 per cent at 650 mμ. Using the percentage of reflection as determined, and the transmission data, he has calculated the absorption for leaves of *Tilia parvifolia* and *Fraxinus excelsior*. The lowest absorption shown for *Tilia* is at 550 mμ, an absorption of 52.7 per cent, and for *Fraxinus* 71 per cent, at the same wave length. He thinks the error in these calculations may not be over 5 per cent, and that they are rather too low than too high.

The interest which had been felt in this problem found no means of expression until April, 1925. At this time a few determinations of the reflection of light from leaf surfaces were made on request by

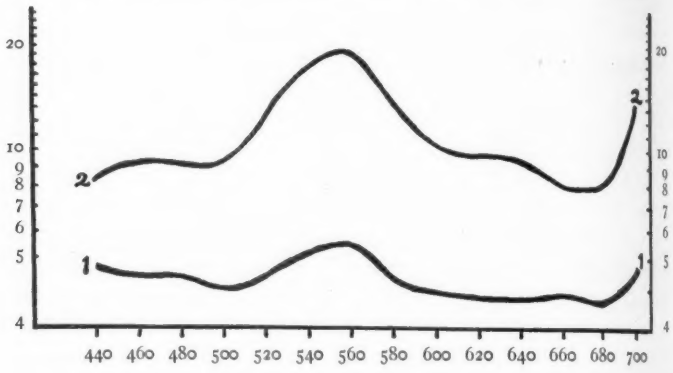

FIG. 1.—Reflection curves for leaves of rhododendron (1), and jonquil (2); note maximum at about 560 mμ, and trough at 680 mμ.

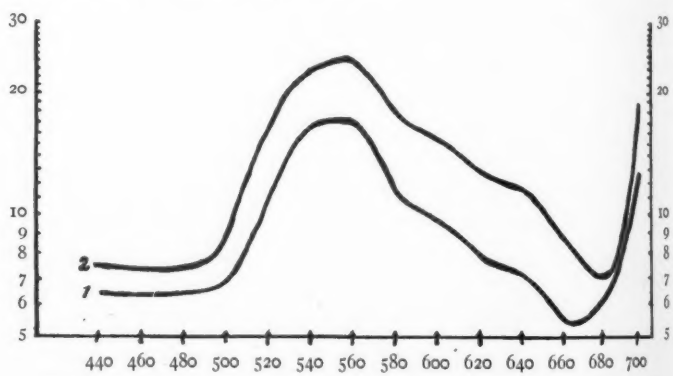

FIG. 2.—Reflection curve for leaves of violet (1), and iris (2); absorption at 660-680 mμ very marked.

the Keuffel and Esser Company, with their direct reading spectrophotometer or color analyzer. The results were of very great interest, and stimulated the making of the extensive series of measurements here recorded. The plotted curves of reflection made by their

observer, Miss MABEL E. BROWN, are shown in figs. 1 and 2. Fig. 1 shows the reflection curve for leaves of rhododendron and of jonquil, plotted on a logarithmic scale. The leaf of rhododendron, being of a darker green than that of jonquil, reflected a much smaller percentage of the incident light at all wave lengths. In both leaves the maximum reflection is near 555–560 mμ, in the brightest of the green portion of the spectrum, agreeing fairly well with POKROWSKI's absorption minimum at about 550 mμ. Fig. 2 shows the curves of reflection for violet and iris leaves. These show a somewhat different form of curve from those in fig. 1, but still the maximum reflection falls at about the same point in the spectrum. The deep depressions in all of the curves between 660 and 680 mμ lie in the region of the main absorption band of chlorophyll. POKROWSKI reported that the strong absorption maximum of chlorophyll at $\lambda = 660$ mμ was not to be seen in his studies. The curves in figs. 1 and 2 certainly show a reflection minimum in that region. In subsequent work this definite depression in the curve at 660-680 mμ was not always seen; however it was frequently observed.

Materials and methods

The leaves of plants of different species vary so much in their superficial physical properties, especially in texture and color, that it is desirable to have a study of the reflection of light from a wide variety of leaves. The leaves of *Verbascum thapsus*, for instance, are so different from those of *Ficus elastica*, that one would expect considerable difference quantitatively in the reflection of light from the leaf surfaces of these plants. Light reflection from vernal vegetation and autumn-colored leaves differs enormously from that from the dark green summer foliage. Even in midsummer foliage, the depth of green color varies widely from species to species, and these differences in color certainly modify the percentage of reflection of incident energy from the surfaces. Leaves discolored by disease or adverse physiological conditions, mildews, infectious or induced chloroses, etc., show increased reflection and decreased absorption of light. Nor do upper and under surfaces of the same leaf reflect equal quantities of incident radiation; in nearly all cases the reflection is considerably greater from the under surface.

On account of this great variability of color and texture, many different kinds of leaves were chosen for this study, but mainly from woody vegetation, trees and shrubs. A partial list of the species used is as follows: *Ulmus americana*, *Betula alba*, *Acer platanoides*, *Syringa vulgaris*, *Psedera quinquefolia*, *Populus alba*, *Prunus pissardi*, *Cersis canadensis*, *Aesculus hippocastanum*, *Sassafras variifolium*, *Castanea dentata*, *Liquidambar styraciflua*, *Catalpa bignonioides*, *Morus rubra*, *Ginkgo biloba*, *Tilia americana*, *Magnolia acuminata*, etc. Among the herbaceous forms, *Zea mays*, *Xanthium italicum*, *Arctium minus*, *Verbascum thapsus*, *Abutilon theophrasti*, *Asclepias syriaca*, etc., may be mentioned. In many of the cases the reflection was measured from both surfaces of the leaves.

The spectrophotometer consists essentially of a prism spectrometer set in front of a spherical box in which two 400 watt lamps are housed. The inside of this spherical box is as white as it can be made. Two blocks of magnesium carbonate serve as reflecting surfaces, and the lamps are so baffled that the light from them does not fall directly upon the reflecting blocks, but is reflected upon them from every possible direction by the curved interior walls of the spherical housing. The reflection is measured perpendicularly to the surface of the blocks of $MgCO_3$, the light coming from these surfaces being directed into the collimator of the spectrometer. After passing through the collimator the light strikes a constant deviation prism, from which it passes to the slit of the telescope. The reflection from the lower block of magnesium carbonate forms the upper half of the field of the telescope, and serves as a comparison field for the reflection from the test object, which is inserted in place of the other $MgCO_3$ block and by its reflection forms the other (lower) half of the field of the telescope. By this arrangement the light from the two spectra are seen side by side, and can be compared as to intensity, just as in other half field instruments, like the MacBeth illuminometer and half field polarimeters.

By means of a wheel, carrying a wave length scale, the constant deviation prism may be adjusted to throw light of any desired wave length into the telescope for comparison. The amount of light coming from the standard block of $MgCO_3$ can be reduced and made equal to that coming from the test object, by means of a rotating sectored double disk. The aperture of the sectors can be changed

while the disks are rotating, by means of a wheel, whose graduated knurled head is scaled to read off the percentage of light transmitted through the revolving sectored disks to the prism. When the two halves of the telescope field show exactly equal illumination at any given wave length, the percentage reading is the percentage of reflection of light at that wave length from the surface of the test object at 90°. The readings are independent of the color vision of the observer, and also independent of the kind of light used to illuminate the specimen under observation. A description of the

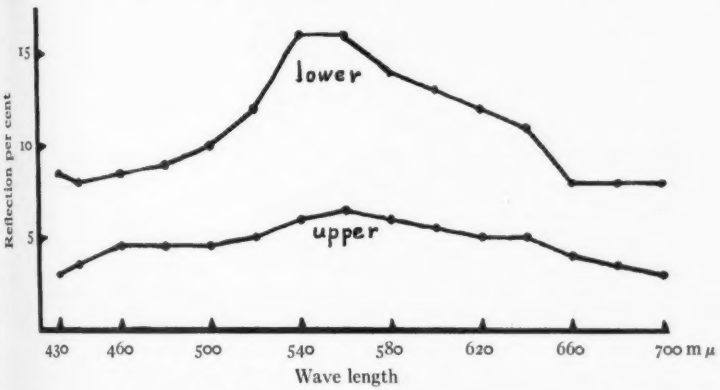

FIG. 3.—Curves of reflection for lilac leaf, comparing upper and under surfaces; curves represent minimum reflection among leaves.

earlier form of this instrument, and directions for its use are given by FERRY (3).

In actual use the upper block of $MgCO_3$ was removed, and the leaf to be measured inserted in its place. The wave length scale on the instrument extends from 430 to 700 mμ, and the reflection was usually measured at 430, 440, and at 20 mμ intervals across the entire spectrum, closing at 700 mμ. The data, in percentage of reflection, are presented in the form of tables and plotted curves.

Results

The reflection data will be shown in several tables, two of which show the general behavior of the late summer dark green foliage, before autumn coloration sets in. Several supplementary tables are

TABLE II

PERCENTAGE OF 90° REFLECTION OF BRIGHT DIFFUSED LIGHT FROM LEAF SURFACES

SPECIES	SURFACE	WAVE LENGTH TESTED														
		430	440	460	480	500	520	540	560	580	600	620	640	660	680	700
Ulmus americana																
Indiana	Upper	5.0	5.5	5.5	6.0	5.0	7.5	10.0	10.0	8.0	7.0	6.0	6.0	6.0	5.5	7.0
	Lower	8.5	11.5	11.5	14.0	14.0	16.0	21.0	20.0	17.0	15.0	15.0	15.0	14.0	14.0	14.0
Minnesota	Upper	4.5	5.0	5.0	5.5	6.0	6.75	9.5	9.0	7.5	7.0	6.25	6.25	6.0	5.25	6.5
	Lower	5.0	5.25	6.0	6.25	7.25	9.5	11.4	11.75	10.5	10.25	9.08	8.5	8.0	6.75	9.75
Acer platanoides																
Indiana	Upper	5.0	5.0	5.0	5.0	5.0	5.5	7.5	8.5	6.5	6.0	5.5	5.0	5.0	5.5	5.5
	Lower	10.0	10.0	11.0	11.0	12.5	16.0	19.0	20.0	16.5	16.5	14.0	14.0	12.5	11.0	15.0
Minnesota	Upper	3.75	3.75	3.75	3.75	4.5	5.0	6.75	6.3	5.0	5.0	4.75	4.25	4.25	4.0	3.75
	Lower	7.0	8.25	8.75	8.75	9.5	12.0	16.0	15.5	13.5	12.25	11.0	10.0	9.0	8.75	11.75
Syringa vulgaris	Upper	3.0	3.5	4.5	4.5	4.5	5.0	6.0	6.5	6.0	5.5	5.0	5.0	4.0	3.5	3.0
	Lower	8.5	8.0	8.5	9.0	10.0	12.0	16.0	16.0	14.0	13.0	12.0	11.0	8.0	8.0	8.0
	Upper	4.5	6.5	6.5	6.5	6.5	7.0	10.0	10.0	9.0	8.0	8.0	8.0	7.5	7.0	6.5
	Lower	11.0	11.0	13.0	13.0	13.0	17.0	21.0	21.0	19.0	17.0	17.0	15.0	15.0	14.0	17.0
Ginkgo biloba	Upper	6.0	5.0	6.0	6.0	6.0	9.0	14.0	15.0	12.5	10.0	10.0	9.5	8.0	6.0	11.5
	Lower	4.5	6.5	8.5	8.5	10.0	13.0	19.0	18.0	16.0	15.0	13.0	11.5	9.5	6.0	13.0
Magnolia acuminata	Upper	5.0	5.0	5.5	5.0	5.0	7.5	12.5	12.5	10.0	6.0	6.0	6.0	6.0	6.0	6.0
	Lower	18.0	20.0	20.0	17.0	18.0	21.0	25.0	23.0	22.5	22.0	19.0	17.5	17.5	16.0	19.0
Liquidambar styraciflua	Upper	6.0	6.0	6.0	6.0	6.0	9.0	11.5	12.0	9.0	8.5	8.0	7.0	6.0	6.0	8.5
	Lower	8.5	8.5	9.5	10.0	10.5	17.0	21.5	21.5	16.0	16.0	13.5	13.5	11.5	11.0	10.5
Catalpa bignonioides	Upper	3.5	3.5	3.5	3.5	4.0	6.5	9.0	11.0	9.0	7.5	7.0	6.5	5.5	5.0	5.0
	Lower	7.0	8.0	12.0	12.0	14.0	16.5	18.0	21.0	20.0	19.0	16.0	15.5	14.0	13.0	12.0
Castanea dentata	Upper	5.0	5.0	5.0	5.5	5.5	7.5	9.0	9.0	7.0	6.0	6.0	6.0	5.0	5.0	6.5
	Lower	8.5	10.0	13.0	13.0	15.0	17.0	19.0	20.5	18.0	17.0	15.5	14.5	12.5	11.0	17.0
Sassafras variifolium	Upper	4.0	4.0	4.0	5.0	5.0	5.0	10.0	10.0	7.0	6.5	6.0	5.0	4.5	4.5	7.0
	Lower	14.0	15.0	20.0	20.0	23.0	25.0	30.0	33.0	29.5	28.0	27.0	26.5	24.0	22.0	27.0
Cersis canadensis	Upper	3.5	4.0	4.0	4.5	5.0	6.0	8.0	9.5	7.0	6.5	6.0	5.5	4.5	5.0	4.0
	Lower	10.0	12.0	13.0	14.0	16.0	21.0	26.5	28.0	27.0	20.0	20.0	20.0	17.0	14.0	19.0
Aesculus hippocastanum	Upper	5.0	5.0	5.0	5.0	5.0	6.0	9.0	10.0	8.0	7.0	7.0	6.5	6.5	6.5	9.0
Populus alba*	Upper	6.5	7.5	9.0	10.0	12.0	14.0	19.0	20.0	16.5	13.0	12.0	12.0	10.5	10.0	8.5

* See also table V for the under surface reflection of *P. alba*.

given, to show the influence of age on the percentage of reflection, the effects of hairiness, the reflection from the surface of albino leaves, the effects of anthocyanin development, and autumn coloration. In table II is shown the general reflection situation with respect to the leaves of trees and shrubs, and in fig. 3 is presented a graph showing the lowest reflection found, in the case of *Syringa vulgaris*.

In table II one sees the ordinary range of variability of green working leaves, from about 6.5 per cent in such forms as lilac and the sycamore-leaved maple, to 20 per cent in *Populus alba*. Some of the under surfaces are even lighter in color, as in *Sassafras*, *Magnolia*,

TABLE III

PERCENTAGE REFLECTION FROM LEAVES OF HERBACEOUS PLANTS

SPECIES	SURFACE	WAVE LENGTHS TESTED (mμ)														
		430	440	460	480	500	520	540	560	580	600	620	640	660	680	700
Asclepias syriaca	Upper	5.0	5.0	5.0	5.0	5.5	7.5	12.0	13.0	9.5	9.0	8.0	7.0	6.5	6.0	5.5
	Lower	10.0	10.0	12.0	11.0	11.5	17.0	20.0	21.5	18.0	17.0	16.0	14.0	13.0	13.0	15.0
Xanthium italicum	Upper	5.5	6.0	6.0	6.0	6.0	13.0	15.0	13.0	10.0	9.0	8.0	7.5	5.0	4.5	8.5
	Lower	8.5	10.0	10.0	10.0	10.0	13.0	18.0	19.0	16.0	12.5	12.0	12.0	11.0	11.0	14.5
Arctium minus	Upper	5.0	5.5	6.0	7.0	7.0	12.0	16.0	16.0	13.5	12.0	10.0	9.5	7.5	7.0	13.0
	Lower	14.0	13.0	13.0	14.0	14.0	19.0	23.0	22.0	19.0	16.0	16.0	14.0	14.0	13.0	17.0
Zea mays	Upper	5.5	6.0	6.5	8.0	8.0	8.0	12.0	13.0	11.5	9.0	9.0	9.0	8.0	7.0	10.0
	Lower	7.0	7.0	8.0	8.0	8.0	11.0	14.0	16.0	14.0	12.0	10.0	10.0	9.0	7.5	12.0

and *Cersis* leaves. In all cases the under surfaces reflect more light than the upper surfaces of the same leaves. This is notably true for the *Sassafras* and *Cersis* leaves. Without exception the maximum reflection falls at 540 or 560 mμ, in the brightest portion of the green region of the spectrum. In the comparison of elm and maple leaves from Indiana and Minnesota, the main difference was that the reflection from the lower surface was less in the Minnesota specimens, indicating that the under surfaces are slightly darker green in Minnesota than in Indiana.

In table III are shown some results with herbaceous plants. These differ in no very striking way from the leaves of woody plants in reflecting power. The depression of the reflection curve in the red is noticeable in *Zea*, *Arctium*, and *Xanthium*, and also in a number of the cases in table II, as in *Ginkgo*, *Castanea*, *Sassafras*, etc.

A few species were found to be developing new leaves as a result

TABLE IV

INFLUENCE OF AGE ON PERCENTAGE OF LIGHT REFLECTION FROM LEAF SURFACES

SPECIES	AGE	SURFACE	WAVE LENGTH TESTED (μμ)														
			430	440	460	480	500	520	540	560	580	600	620	640	660	680	700
Tilia americana	Young	Upper	7.5	8.0	8.0	9.0	14.0	21.0	27.0	28.0	24.5	23.0	20.0	19.0	16.0	10.0	20.0
	Medium	Upper	3.0	6.0	6.5	5.0	5.0	10.0	15.0	15.0	12.0	10.0	8.0	8.0	5.0	4.0	9.0
	Old	Upper	5.0	4.0	6.0	6.0	5.0	7.0	10.0	10.0	8.0	7.0	6.0	6.0	6.0	4.0	7.5
Populus deltoides	Young	Upper	3.0	3.0	5.0	6.5	6.5	12.0	21.0	22.0	21.0	16.0	14.0	12.5	9.0	7.0	6.0
	Old	Upper	4.5	5.0	5.5	6.5	8.0	10.0	14.0	16.0	12.0	11.0	9.0	8.0	7.5	7.0	6.5
Ficus elastica	Young	Upper	4.0	4.0	4.0	4.0	5.0	7.0	11.0	11.0	11.0	10.5	10.0	9.5	6.5	5.5	10.0
		Lower	6.0	8.5	9.5	10.0	10.0	14.0	15.0	17.0	18.0	19.0	18.5	18.0	13.0	11.0	17.0
	Old	Upper	3.0	3.0	4.0	5.0	5.5	7.5	12.0	11.5	9.0	6.5	5.5	4.5	4.5	4.5	5.5
		Lower	9.5	10.5	11.0	11.0	11.0	18.0	24.0	24.0	22.0	20.0	17.0	15.0	13.0	10.0	15.0
Cersis canadensis	Young	Upper	3.5	4.5	5.0	5.0	5.0	6.0	9.0	9.0	7.5	6.5	5.5	5.0	4.5	3.5	4.0
	Old	Upper	3.5	4.0	4.0	4.5	5.0	6.0	8.0	9.5	7.0	6.5	6.0	5.5	4.5	5.0	4.0

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of copious rains during the later part of the summer, 1926. Comparative measurements, of upper surfaces mainly, were made of leaves of different age in four species, *Tilia americana*, *Cersis canadensis*, *Populus deltoides*, and *Ficus elastica*. The data are presented in table IV, and the results with *Tilia americana* are shown graphically in fig. 4. In fig. 4 the curve marked "medium" was made

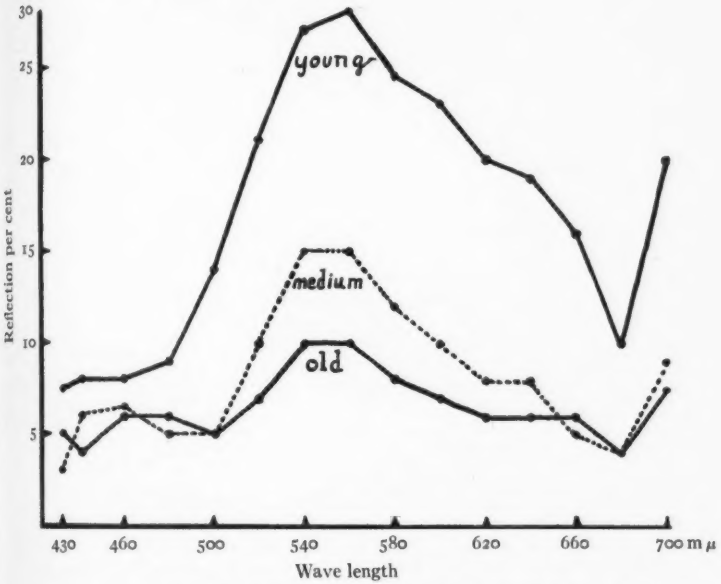

FIG. 4.—Curves of reflection showing effects of aging on leaves of *Tilia americana;* chlorophyll development brings decreased reflection.

from a leaf not much older than the young leaf, possibly only a week or so older. It was intermediate in shade of green to the eye, when seen with the young and old leaf. The old leaf was one which had been functioning all summer. The leaves must change very rapidly from the condition of spring verdure to the full dark green of summer, and yet POKROWSKI (4) shows that leaves at 1.5 months have not yet reached the point of least reflection.

Some attention was given to hairiness, since it was customary formerly to attach some importance to it as an ecological factor.

SAYRE (5) showed some years ago that hairiness had little significance in the process of transpiration. Leaves of *Verbascum*

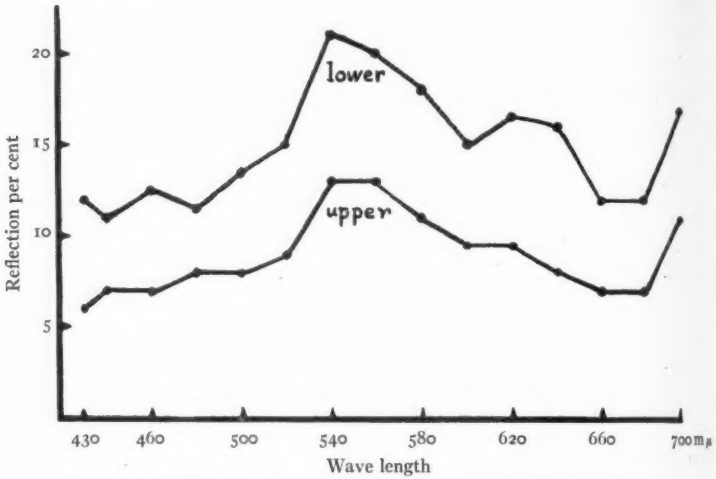

FIG. 5.—Reflection from leaves of *Verbascum thapsus;* in this case hairs have little effect on amount of reflection.

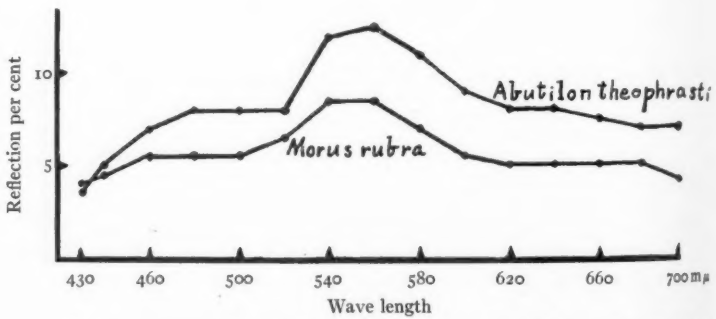

FIG. 6.—Comparison of reflection from upper surface of *Abutilon theophrasti* and *Morus rubra;* neither hairs nor shiny cuticle increase reflection in these cases.

thapsus and *Abutilon theophrasti* were chosen for the presence of hairs, and *Morus rubra* for its very shiny smooth cuticle. The red mulberry leaf has the appearance of having been varnished on its

TABLE V

INFLUENCE OF HAIRINESS ON PERCENTAGE OF LIGHT REFLECTION FROM LEAF SURFACES

SPECIES	LOCALITY	SURFACE	WAVE LENGTH TESTED (Mμ)																
			430	440	460	480	500	520	540	560	580	600	620	640	660	680	700
Verbascum thapsus	Indiana	Upper	6.0	7.0	7.0	8.0	8.0	9.0	13.0	13.0	11.0	9.5	9.5	8.0	7.0	7.0	11.0
		Lower	12.0	11.0	12.5	11.5	13.5	15.0	21.0	20.0	18.0	15.0	16.5	16.0	12.0	12.0	17.0
	Minnesota	Upper	5.5	5.5	6.3	6.3	6.5	9.0	12.5	13.0	10.5	9.3	8.0	7.8	6.5	6.3	9.3
		Lower	10.5	11.0	11.5	12.3	14.0	16.5	21.8	20.5	18.0	16.8	15.8	15.0	14.0	11.8	14.3
Abutilon theophrasti	Indiana	Upper	3.5	5.0	7.0	8.0	8.0	8.0	12.0	12.5	11.0	9.0	8.0	8.0	7.5	7.0	7.0
		Lower	10.0	11.5	13.0	13.0	13.0	17.0	19.0	17.0	15.0	14.0	13.0	12.0	10.0	10.0	11.0
Morus rubra	Indiana	Upper	4.0	4.5	5.5	5.5	5.5	6.5	8.5	8.5	7.0	5.5	5.0	5.0	5.0	5.0	4.0
		Lower	7.5	8.0	8.0	9.0	10.0	12.5	14.0	15.0	13.0	11.0	10.0	10.0	9.0	7.5	10.0

upper surface. The reflection from these leaves is shown in table V, and fig. 5 records in graphic form the difference in reflecting power of the upper and lower surfaces of the Indiana specimen of *Verbascum thapsus*. In fig. 6 is shown the reflection from the upper surface of the velvet leaf, *A. theophrasti*, and the red mulberry. The very shiny surface of the latter reflects less light by 4–5 per cent than the hairy surface of *Abutilon*, when measured at 90° to the surface. On the other hand, the reflection from the mullein leaves is no greater than that for many smooth leaves. The hairs must have very little influence on the light relations. SAYRE showed that

TABLE VI

INFLUENCE OF WHITE SURFACES AND ALBINISM ON PERCENTAGE OF LIGHT REFLECTION

SPECIES	SURFACE	WAVE LENGTHS TESTED (Mμ)														
		430	440	460	480	500	520	540	560	580	600	620	640	660	680	700
Populus alba	Upper	6.5	7.5	9.0	10.0	12.0	14.0	19.0	20.0	16.5	13.0	12.0	12.0	10.5	10.0	8.5
	Lower	50.0	50.0	50.0	50.0	50.0	51.0	50.0	52.0	53.0	51.0	52.0	51.0	50.0	51.0	50.0
Geranium																
Green	Upper	6.0	7.0	10.0	7.0	8.5	12.0	16.0	15.0	12.0	12.0	11.0	10.5	10.0	10.0	9.5
Albino	Upper	25.0	31.0	31.0	33.0	40.0	43.0	43.0	47.0	45.0	45.0	45.0	45.0	45.0	45.0	43.0
Syringa vulgaris																
Healthy	Upper	3.0	3.5	4.5	4.5	4.5	5.0	6.0	6.5	6.0	5.5	5.0	5.0	4.0	3.5	3.0
Mildewed	Upper	9.0	12.5	12.5	13.0	12.5	13.0	13.5	16.0	14.0	12.5	12.0	13.0	15.0	15.0	16.0

transpiration from these leaves is controlled by stomatal action, just as in non-hairy leaves. COBLENTZ also found that *Verbascum* leaves are much like others in reflecting power, notwithstanding their hairy surface. The leaf of *Verbascum* shows a low reflection in the region of the red absorption band of chlorophyll, as shown in fig. 5. This is not evident, however, in the measurements of *Abutilon* and *Morus* shown in fig. 6. For a case where tomentose hairiness does affect reflection see table VI, the under surface of the leaf of *Populus alba*.

Some leaves have very white surfaces, as has just been mentioned for *P. alba*, and in a few instances we have albino plants, or plants with occasional albino leaves. The common white-edged geranium (Madame Seleroy) bears such leaves, without chlorophyll. Whitening of the surface may result from the presence of mildew or other

FIG. 7.—Reflection curves for tomentose under surface of *Populus alba* leaf, and for upper surfaces of green and albino leaves of geranium (Madame Seleroy); note uniform reflection of gray color, also smaller reflection in blue region from albino leaves.

fungi, as in the case of the lilac whose leaves at the end of summer are frequently covered with the mycelium of *Microphaera alni*.

Measurements of both surfaces of the *P. alba* leaves, of green and albino geranium, and the upper surfaces of healthy and mildewed lilacs are presented in table VI.

The extraordinary situation with respect to the lower surface of *P. alba* is just what one would expect on the basis of its color. It looks very white to the eye, but should no doubt be considered as

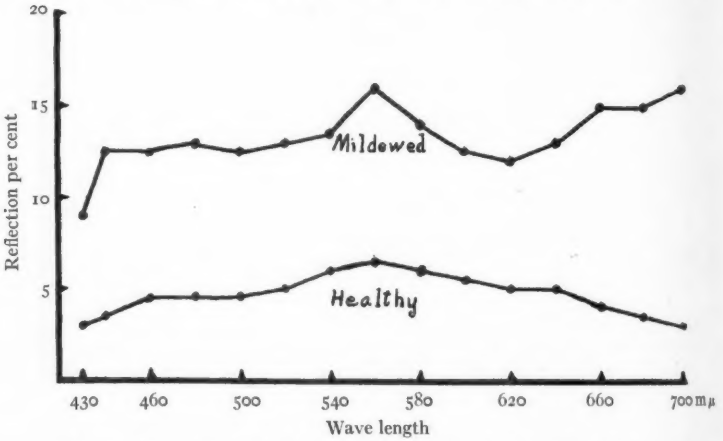

FIG. 8.—Comparison of reflection from healthy and mildewed lilac leaves

gray. The reflection is almost the same across the entire spectrum, usually slightly above 50 per cent. The whiteness is due to the closely matted woolly covering. In fig. 7 is shown graphically the reflection curve for the lower surface of *P. alba*, and a comparison of the green and albino geranium leaf reflection (upper surface in both cases).

The peculiar reflection curve of the albino geranium leaf, low in the blue region as compared with the red portion, is associated with the yellow color of the albino leaves. The yellow color of the leaf is due to the absorption of the blue radiation; and if the reflection were as great from 430 to 560 mμ as elsewhere in the spectrum, one would expect the albino leaf to be almost as white as the under surface of the leaves of *P. alba*.

The effects of the mildewing of lilac can be seen at a glance in fig. 8. The reflection is 2–3 times as great from the diseased as from the healthy leaf. That the light relations are unfavorably affected is not a new idea, but this is probably the first quantitative measure of the effect.

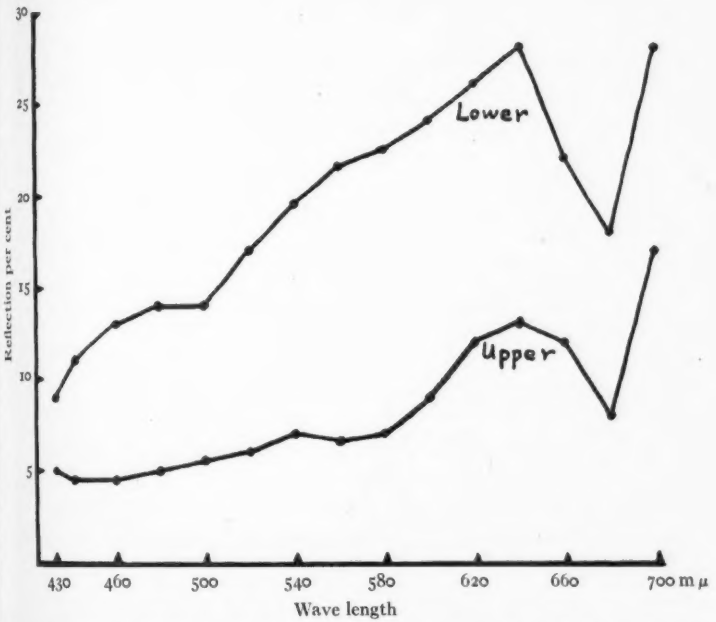

FIG. 9.—Reflection curves for autumn-colored *Psedera quinquefolia* leaves; note reflection peak at 640 and depression at 680 mμ.

The influence of anthocyanin development on reflection was observed in several leaves. The measurements concern *Prunus pissardi*, the lower surface of the leaves of *Acer palmatum atropurpureum*, and the crimson autumn-colored leaves of *Psedera quinquefolia*. The reflection data from these leaves are shown in table VII, and graphs for *Psedera* leaves are given in fig. 9. The maximum reflection is shifted toward the red region of the spectrum, but with a very sharp depression of the curves at 680 mμ. The lower surface is seen to reflect several times as much light as the upper one. A similar

situation is seen in the case of lower surface reflection from the leaf of *Acer palmatum atropurpureum* from 600–700 mμ. The upper surface of this leaf has no anthocyanin, and is in sharp contrast to the lower surface (table VII). The *Prunus pissardi* leaf shows only a slightly higher reflection in the red region than in other portions of the spectrum. Its reflection is lower than that for many other species.

A small number of readings were made of brilliant yellow autumn-colored leaves, particularly of *Betula alba*, and the upper surface of *Populus deltoides*. The data are presented in table VIII,

TABLE VII

INFLUENCE OF ANTHOCYANIN DEVELOPMENT ON PERCENTAGE OF REFLECTION OF LIGHT FROM LEAVES

SPECIES	SURFACE	WAVE LENGTHS TESTED (mμ)														
		430	440	460	480	500	520	540	560	580	600	620	640	660	680	700
Prunus pissardi	Upper....	5.0	5.0	4.5	4.5	5.5	6.0	7.0	6.0	6.0	8.0	8.0	7.0	6.0	5.5	8.0
Acer palmatum atropurpureum	Upper....	4.0	5.0	5.0	5.0	5.0	5.5	6.5	7.0	6.0	5.0	5.0	5.0	4.5	5.0	5.0
	Lower....	6.0	7.5	8.0	8.0	7.0	8.0	8.0	8.0	9.0	10.5	12.0	14.0	14.0	11.0	13.5
Psedera quinquefolia	Upper....	5.0	4.5	4.5	5.0	5.5	6.0	7.0	6.5	7.0	9.0	12.0	13.0	12.0	8.0	17.0
	Lower....	9.0	11.0	13.0	14.0	14.0	17.0	19.5	21.5	22.5	24.0	26.0	28.0	22.0	18.0	28.0

and the curves for the *Betula* leaves in fig. 10. These remarkable curves, like the curve for the albino geranium leaf in fig. 7, show low reflection in the blue region, and very high reflection in the red end of the spectrum. The upper surface reflection reaches a maximum of 42 per cent, agreeing rather well with COBLENTZ's forty-five degree readings for *Liriodendron tulipifera* on October 31 (table I), in which he found 47–48 per cent of the light reflected.

The data here presented, taken as a whole, give one a rather general picture of the light reflection situation as a factor in the energy relations of leaves. The significance of the data will be considered briefly in the following section.

Discussion

From the work of COBLENTZ, POKROWSKI, and that here reported, it is evident that a very considerable portion of the visible radiant energy falling upon the leaf is reflected from it. Some of this

reflection is directly from the cuticular surface, and some undoubtedly from reflecting interior surfaces, the light having penetrated, but

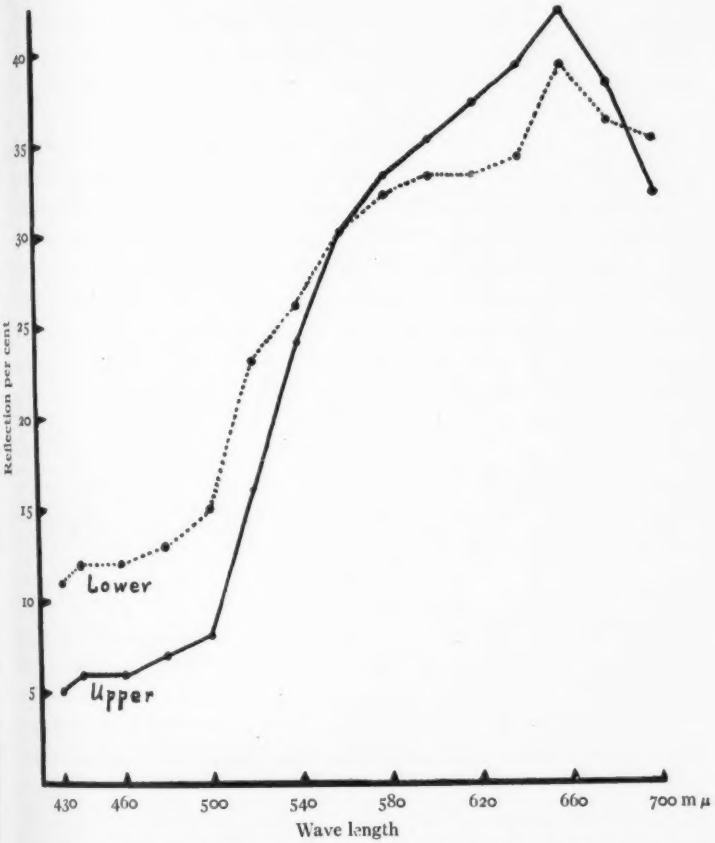

FIG. 10.—Reflection curves for autumn-colored leaves of *Betula alba;* low reflection in blue region is notable in yellow coloration.

being reflected from within. It does not really make much difference whether the reflection is external or internal, for the reflected energy does no internal work, and cannot be transmitted. It must all be subtracted from the total incident energy, just as we subtract

the transmitted energy when we attempt to determine the total absorption of radiation.

The principal value of this work lies in the fact that it shows the magnitude of the reflection factor in the region of visible light. This magnitude is so great that it cannot be neglected in an attempt to establish an energy account for the leaf. It is valuable also in emphasizing the variation of reflection with species. The BROWN and ESCOMBE studies were made only on *Catalpa bignonioides*, which is shown in table I to reflect 6 per cent of the incident light; 11 per cent at maximum in the green. A great many leaves reflect a larger proportion of the light. Data from *Catalpa* alone could hardly be accepted as representative of the entire plant kingdom. The leaf of

TABLE VIII

INFLUENCE OF AUTUMN YELLOW COLOR ON REFLECTION OF LIGHT FROM LEAVES

SPECIES	SURFACE	WAVE LENGTHS TESTED (Mμ)														
		430	440	460	480	500	520	540	560	580	600	620	640	660	680	700
Populus deltoides	Upper....	8	9	10	11	11	24	33	38	42	40	39	38	38	33	34
Betula alba	Upper....	5	6	6	7	8	16	24	30	33	35	37	39	42	38	32
	Lower....	11	12	12	13	15	23	26	30	32	33	33	34	39	36	35

Catalpa is thin, and many other types of leaves may have a larger absorption coefficient because they are thicker or more deeply pigmented. POKROWSKI found the absorption coefficient to vary from 52 to 95 per cent of the total.

Thickness of the leaf would also become a factor of importance in the thermal emissivity relations. Here again the *Catalpa* leaves are not to be considered as representative, although there are many thin-leaved plants. The absorption coefficient for *Catalpa* given by BROWN and ESCOMBE is probably too large, since reflection was counted in as a part of the absorption. If this is true, then their figures for the internal work must be on the whole too large also.

If there were any simple way in which a correction factor for reflection could be applied to each of the energy changes in internal work, the matter would not be serious, but there is no way in which this can be done. Therefore it seems that the whole question of

energy relations must be reopened and investigated anew, to determine the energy relations for every significant process. This should be done with a wide range of material so that the ordinary limits of variability may be known.

It should be acknowledged at once that, valuable as these spectrophotometric studies are, they are not in themselves sufficient for the revision of the energy relations with respect to reflection. These studies do show that reflection is a large factor and that revision is necessary; but the inadequacy of the spectrophotometer for the ultra-violet and infra-red reflection is obvious. We have measured only the *visible* reflection. While the reflection in the ultra-violet would be small in any event, the infra-red reflection is large. COBLENTZ found 38 per cent reflection at 950 mμ from green leaves of *Liriodendron*. At 4400 mμ it was still 5.6 per cent. To make a proper reflection correction would require a complete study of the spectrum from about 290 mμ to the far limits of the infra red. Absorption should also be studied directly, if possible, with various types of leaves, so that a more accurate balance of the energy changes might be established. Because of the limitations of the spectrophotometer method, it may be necessary at least to supplement it by the use of COBLENTZ'S method for exploring the invisible regions of the solar spectrum.

Summary

1. The reflection of light from the surfaces of many kinds of leaves has been measured by means of a direct reading spectrophotometer, the measurements being made normal (that is, at an angle of 90°) to the leaf surface. The incident light, falling upon the leaf from the surface of a spherical housing, strikes the leaf at all angles.

2. The amount of reflection varies with the wave length, the maximum reflection falling usually at 540–560 mμ in green leaves. The value of the reflection in this region runs from 6–8 per cent in the darkest green leaves to 20–25 per cent in the lightest green specimens.

3. Hairiness or smoothness of cuticle does not necessarily mean high reflection. Leaves of *Verbascum thapsus* and *Abutilon theophrasti* show very little more reflection than leaves of non-hairy

plants. The shiny cuticle of *Morus rubra* adds little to normal reflection. In some cases, however, as in the under surface reflection of *Populus alba* and *Magnolia acuminata* leaves, the hairs do increase the reflection.

4. The amount of reflection decreases with the age of the leaf. This is associated with the development of chlorophyll, which increases in amount very rapidly at first, and then more gradually for about two months, until the final depth of green color has been attained. The reflection then remains unchanged until the beginning of chlorophyll destruction at the close of summer.

5. White surfaces reflect almost equally across the spectrum, as seen in the under surface reflection of *Populus alba;* while albino leaves reflect mainly the longer radiations. There is absorption of the blue rays, corresponding to the yellowish color. The reflection from such surfaces is 40–50 per cent of the incident radiation.

6. The presence of mildew or other whitish superficial organisms increases the reflection of light very noticeably. In lilac the presence of *Microsphaera alni* increased the reflection more than 100 per cent.

7. Anthocyanin development is accompanied by a shift in the position of maximum reflection to the longer wave lengths. In *Psedera* the maximum reflection occurred at 640 mμ, while the normal maximum for green leaves is 540–560 mμ.

8. Yellow autumn coloration is much more brilliant than red. The reflection of yellow *Betula* leaves reached 42 per cent, with the maximum at 660 mμ. A leaf of *Populus deltoides*, less completely yellowed, reflected as much light, but the maximum reflection was in the yellow, at 580 mμ, instead of in the red.

9. In a considerable number of cases there is a depression of the reflection curve at 680 mμ. This obviously corresponds to the maximum absorption band of chlorophyll. The fact that it is evident is an argument for reflection from internal surfaces, in part.

10. The data presented are valuable in connection with the problem of income and outgo of energy in the leaf processes; but they must be supplemented with measurements in the invisible regions of the spectrum, particularly in the infra-red region, before a complete accounting of the energy utilization can be rendered.

It is a pleasure to record indebtedness to the Keuffel and Esser

Company for their interest in this work, and also to Professor ERVIN S. FERRY, of the Department of Physics, Purdue University, in whose laboratory many of these measurements were made in September, 1926. Acknowledgment is likewise made to Professor CLYDE H. BAILEY, of the Department of Agricultural Biochemistry, University of Minnesota, in whose laboratory some comparative tests were run in October, 1926, with a more recent model of the spectrophotometer. A few measurements of highly colored autumn yellow leaves were made at Minnesota also. The spirit of cooperation and helpfulness shown in these laboratories is gratefully recorded.

UNIVERSITY OF CHICAGO

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MEIOTIC PHENOMENA IN CERTAIN GRAMINEAE[1]

I. FESTUCEAE, AVENEAE, AGROSTIDEAE, CHLORIDEAE, AND PHALARIDEAE

GEORGE L. CHURCH

(WITH PLATES XXV–XXVII)

Introduction

The statement of CHARLES DARWIN that the facts of variation under hybridization did not seem to him opposed to the belief that "species aboriginally existed as varieties" is indeed prophetic, in the light of the research of the last two decades. ROSENBERG'S work on a natural *Drosera* hybrid (43), involving a cross between parents of different chromosome complements, clearly demonstrated the correlation between cytological abnormalities of the pollen mother cells during the maturation divisions and the sterile pollen resulting from such crossing of species. Similar and even more involved cytological conditions have been found to be widespread among the species of many taxonomically variable plant groups. The researches on *Hieracium* (ROSENBERG 44), *Rosa* (TÄCKHOLM 47), *Rubus* and *Crataegus* (LONGLEY 32, 33), and *Viola* (CLAUSEN 6) are outstanding examples. LOTSY (37) states:

> While DE VRIES in his mutation theory predicted that the cause of the polymorphy in *Oenothera*, *Draba* and *Viola*, as well as the nebulae of the older systematicists in *Rubus*, *Hieracium*, *Rosa* and *Salix*, would prove to be due to mutation, we now know that in all these cases . . . . hybridization is responsible for by far the greater part of that polymorphy.

In view of the importance of hybridization in the multiplication of species in a host of large genera and families, a cytological investigation of the pollen mother cells of typical representatives of such a large and widespread family as the Gramineae has been considered capable of yielding further significant evidence in this direction.

The hybrid origin of many of our cereals has already been demonstrated by careful genetical and cytological research. Very complete

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

work has been done on *Triticum* by SAX (45). The wheats fall into three definite polyploid groups, diploid, tetraploid, and hexaploid. SAX considers that the differential mating of chromosomes in crosses between these groups indicates a probable hybrid ancestry for the polyploid members. PERCIVAL (41) notes the extreme antiquity of all the *Triticum* groups, certainly necessitating a very remote period of hybridization. His work on *Aegilops* leads him to the opinion that in this genus may be found one of the ancestors of the "vulgare" or hexaploid wheats.

The same polyploid groups occur in *Avena* as occur in *Triticum*. HUSKINS (24) finds that our commonest oat species, *A. sativa*, often gives rise to so-called "fatuoid" types with aberrant chromosome complements:

> The ordinary practically awnless type of *A. sativa* is considered as being the resultant of diverse factors present in its constitution on account of its polyploid and probable hybrid origin.

COLLINS (8) maintains that the cultivated *Zea mays* is of hybrid origin, involving a cross between annual Teosinte (*Euchlaena mexicana*) and some species of *Andropogon*. COLLINS' supposition has been substantiated by the cytological researches of KUWADA (31) and LONGLEY (34). VINALL (52) infers a hybrid lineage for our cultivated sorghums as a result of cross experiments with wild sorghum and Johnson grass.

There are many indications that hybrid grasses exist in addition to the cultivated forms. BREMER (4) considers that the polyploidy of many members of the Andropogoneae allied to *Saccharum* is due to hybridization. EVANS (13) has made chromosome counts in species of *Lolium* and *Festuca* and notes indications of polyploid series similar to those in the cereals. TURESSON (51), in a taxonomic study of the variable *Festuca ovina*, notes normal sexual, facultative and wholly viviparous types in Sweden in a series progressing northward. He suggests that the sterile types are a result of crossing, and compares them with the apomictic *Hieracium* forms in which hybridization has been firmly substantiated. That the sterile pollen and extreme variability of the dichotomum section of the genus *Panicum* indicate hybridity has been accepted very generally. JEFFREY (26) notes a considerable percentage of bad pollen in the common *Alop-*

ecurus pratensis. COCKAYNE (LOTSY 38) lists many systematically recognized hybrid grasses growing in New Zealand; hence the need of cytological data concerning some of our common grasses is evident.

Materials and methods

The grasses examined in this research were collected from various park areas and reservations about greater Boston. Some few exotic species were obtained in the Botanic Gardens of Harvard University and in waste land bordering railroads. Collecting was limited to the early afternoon hours of warm days during the season when the pollen is maturing. A special effort was made to collect those species that revealed bad pollen upon examination of material from the collecting season of the previous year. Carnoy's fluid was employed as a killing and fixing agent, and was pumped in with a field exhaust pump to insure rapid penetration.

The nature of the open panicle type of inflorescence found in many grasses would naturally make the imbedding of a quantity of spikelets exceedingly difficult if there were not some means of concentrating the latter in a compact group. The "glycerin-jelly" method for imbedding small objects in mass as developed in the laboratories of plant morphology of Harvard by Professor E. C. JEFFREY has been employed with complete success in this work. The material was imbedded in nitrocellulose, and 5μ sections were made on a Jung-Thoma sliding microtome. The usual Haidenhain's iron-alum haematoxylin stain was employed, with a weak solution of eosin in 30 per cent alcohol for cytoplasmic contrast. Preparations were studied with a Zeiss 1.5 mm. apochromatic or a Bausch and Lomb 97× oil immersion objective and a no. 12 compensating ocular. Drawings were made by the aid of a Bausch and Lomb camera lucida and a stage micrometer to calculate the magnifications.

Cytology of species

The sequence of the tribes and the nomenclature follow the treatment of HITCHCOCK (21). Revisions appearing subsequent to his publication and appearing in the files of the Gray Herbarium of Harvard University are indicated. The present article deals with species studied in the Festuceae, Aveneae, Agostideae, Chlorideae,

and Phalarideae. A second article will deal with species in the Paniceae and Andropogoneae.

Festuceae

Festuca ovina L., Sheep Fescue (pollen 10–20 per cent imperfect).—Chromosome counts of diakinesis stages and heterotypic equatorial plates reveal the octoploid number of twenty-eight bivalents (fig. 8). Many of the divisions are quite regular, resulting in normal tetrads and pollen; however, not infrequently unpaired mates are found in diakinesis. These chromosomes may be extruded as univalents into the cytoplasm during heterotypic metaphase stages, the latter often presenting lagging bivalents as well (fig. 6). The following anaphase in even the last stages shows lagging bivalents (fig. 7). These laggards persist during the diad stage (two cells in a common gelatinous matrix at the end of the heterotypic division), and during the homeotypic division, with the resultant production of aberrant nuclei (polycary) in the tetrads and consequent abortive pollen grains.

Festuca rubra L., Red Fescue (pollen about 25 per cent imperfect).—Diakinesis shows twenty-one ringed bivalents (fig. 9). Equatorial plates also reveal the hexaploid number. Cytomyxis may occur in the prophase stages, often resulting in the persistence of chromatin in the cytoplasm during subsequent divisions. Chromosomes may often become stranded in adjacent mother cells during diakinesis (fig. 9), and give rise to the extrusions found in the following reduction division (fig. 10). The latter figure also shows the common tendency of some bivalents to separate before others and become stretched over the spindle. Such irregular disjoining of the bivalents accounts for the laggards in the early anaphase stages. The homeotypic divisions are quite normal, and extruded chromatin seems to be dissipated by the time the tetrad stage is reached.

Festuca duriuscula L., Hard Fescue; a European introduction collected in the Harvard Botanic Garden plots (pollen 60–70 per cent imperfect).—This species is also hexaploid, as shown by the twenty-one bivalents at the equatorial plate (fig. 11). Laggards and extruded chromosomes are common in the heterotypic metaphase (fig. 1) and anaphase (fig. 2). Frequently one or two bivalent pairs separate in advance of the others, giving the irregular appearance noted

in *F. rubra*. Extruded chromatin, obviously in the path of obliterated spindle fibers, often appears even when the mother cell is nearly split at the equator to form the diad stage (fig. 4). Chromatin has been observed to bridge even the gap in this split. The homeotypic divisions are normal (fig. 5), but extrusions from the heterotypics may persist and give rise to polycaric tetrads. The phenomenon of cytomyxis is not uncommon at the early interkinesis stage (fig. 3).

Dactylis glomerata L., Cocksfoot or Orchard Grass; naturalized from Europe (pollen 40–50 per cent imperfect).—The tetraploid complement of fourteen bivalents published by DAVIES (10) is confirmed by examination of this material. Although the divisions are not generally characterized by lagging or extruded chromatin, the phenomenon of non-pairing is rather conspicuous. Late diakinesis, characterized by a large nucleus and a much vacuolated nucleolus, often shows twenty-eight univalents. These univalents pair tardily just before the spindle appears, but loosely associated pairs may be seen in the early heterotypic metaphase. Diakinesis stages of part bivalent and part univalent chromosomes may also be observed. Polar views showing unpaired mates at the plate are of frequent occurrence. The large percentage of imperfect pollen in the absence of polycary is striking.

AVENEAE

Avena sativa L., common oats; an escape from cultivation and conforming closely to *A. sativa* L. var. *mutica* Al. according to KOERNICKE (in CARLETON 5) (pollen normal).—The twenty-one bivalents at the equatorial plate (fig. 13) mark the strain as hexaploid, corresponding to all the types of *A. sativa* investigated (HUSKINS 24). Normal divisions and tetrads are found, but many outstanding irregularities make their appearance. Lagging bivalents in the form of rings appear in both early (fig. 12) and late (fig. 14) heterotypic metaphases. Likewise lagging univalents are as frequent in the corresponding stages of the anaphase (figs. 15, 16). Occasionally an extreme case of lagging and extrusion may be noted in the telophase (fig. 17). The chromosomes are quite large, and often the homeotypic split can be seen anticipated as a notched groove in the disjoined univalents. Laggards again appear in both stages of the homeotypic division (figs. 18, 19). The homeotypic telophase (fig.

20) is marked by extrusions closely aggregated about the nuclei, which result in a high degree of polycary in the tetrads (fig. 21). The normal pollen is in surprising contrast to such conditions in the maturation divisions.

AGROSTIDEAE

Ammophila breviligulata Fern. The species of the eastern United States coast has been shown (FERNALD **15**) to be quite distinct from the European *A. arenaria* (L.) Link in its much larger spikelike panicles and truncated ligule (pollen 40–50 per cent shriveled).—The tetraploid number of fourteen bivalents may be counted in the diakinesis stage, or in polar view of the equatorial plate. Cytomyxis is observed at diakinesis in some instances. Mother cells in a complete state of collapse may be found as a result of excessive chromatin loss. These appear in the same anther sac with normal mother cells. Laggards are not uncommon in the heterotypic anaphase, all other divisions being quite normal. Extrusions resulting from lagging and probably also from cytomyxis may persist in the cytoplasm of the mother cells throughout the second division and give rise to polycaric tetrads.

Alopecurus geniculatus L. var. *aristulatus* Torr. According to GRAY'S manual this variety is introduced from Europe in the eastern United States, but blends into the indigenous *A. geniculatus* L. in the western states (pollen 30–40 per cent imperfect).—This is one of the few grasses found in this research with the diploid complement of seven bivalents. The chromosomes are comparatively large, and appear clearly as rings in diakinesis, in which stage they show also a decided tetrad structure. Cytomyxis occurs at prophase, diakinesis, and occasionally at the early heterotypic metaphase. In the last two stages whole chromosome pairs are seen stranded between two mother cells. Laggards in the early heterotypic metaphase usually persist as such in the late metaphase. The early anaphase is frequently marked by a partly split bivalent that stretches along the spindle in advance of the other pairs. One or more tardily splitting bivalents may accordingly be seen in the late anaphase, and extruded into the cytoplasm in the telophase. Occasionally lagging chromosomes appear in both the homeotypic metaphase and anaphase.

Laggards from either division may be extruded and cause aberrant nuclei to form in the tetrads (polycary).

Alopecurus pratensis L., Foxtail Grass; naturalized from Europe (pollen 20–30 per cent imperfect).—Diakinesis reveals fourteen ringed bivalents, making this a tetraploid species. This confirms the number reported by MARCHAL (**39**). Cytomyxis is frequent in occurrence in the spireme and diakinesis stages. Excessive chromatin loss may result in the disintegration of the mother cell involved, as noted in the case of *Ammophila* and *Festuca duriuscula*. Bivalents at the plate but with half of the ring stranded in the cytoplasm of a closely adjacent cell are noted occasionally in the first division. Unpaired mates may be found in diakinesis which lag as univalents on the heterotypic spindle. Such univalents are either extruded into the cytoplasm or are included in the telophase nuclei, remaining at the poles during the metaphase. The nucleolus has been seen stranded on the spindle in a few instances. The heterotypic anaphase is often marked by laggards which result in a prominent streak of chromatin stretched between the telophase nuclei similar to the condition figured for *Festuca duriuscula* (fig. 4). The extrusions from the heterotypic division usually persist throughout the homeotypic, which may less frequently present laggards and extrusions. Polycary is not uncommon.

CHLORIDEAE

Spartina michauxiana Hitch. (pollen 30–40 per cent imperfect).—This is a tetraploid species, the diakinesis clearly showing fourteen bivalents (fig. 29). The heterotypic division (figs. 30, 31) proceeds in quite regular fashion, as does the homeotypic. Only rarely does a chromosome become extruded during the heterotypic anaphase. All stages seem to be marked by cytoplasmic chromatin, which persists frequently and causes the appearance of polycaric, vacuolated tetrads (fig. 32). So far as the investigated material is concerned, the cytoplasmic chromatin seems to owe its presence in part to chromatin interchange at cytomyxis which may occur in the prophase stages.

Spartina alterniflora Loisel. var. *glabra* (Muhl.) Fern.[2] (pollen

[2] See FERNALD (**14**) for revision of species names.

40–50 per cent imperfect).—Careful examination of the diakinesis stage reveals fourteen bivalents (some loosely paired) and fourteen univalents, the somatic total making the species hexaploid (fig. 22). It will be noted that at even the late stage of the diakinesis figured, as is evidenced by the vacuolated nucleolus, the univalents still retain remnant, threadlike connections; hence they are not clearly delineated in earlier stages because of their persistence in the spireme. These univalents are extruded freely into the often vacuolated cytoplasm during the heterotypic metaphase, some few remaining on the spindle (fig. 23). Occasionally some of the univalents may split at the plate with the bivalents, as is evidenced by the polar view of the metaphase (fig. 24). It is exceptional to find apparently all of the univalents on the spindle, however, as is figured in this diagram. Cytomyctic strands, facilitating the passage of chromatin between mother cells, may also be seen during the heterotypic division (fig. 25). The bivalents move quite regularly to the poles in the anaphase, the univalents or split univalents, if there are any persistent, being distributed at random (fig. 26). This figure also shows many of the extruded univalents coalescing into larger masses preparatory to disintegration in the cytoplasm. There is no splitting of the univalents included in the nuclei during the homeotypic division (fig. 28), as is evidenced by a comparison of a polar view of the homeotypic metaphase with that of the heterotypic (fig. 24). These univalents still lag on the spindle, however, the bivalents proceeding in normal fashion (fig. 27). Extruded chromatin still present in the cytoplasm at this stage causes the not infrequent appearance of polycary in the resultant tetrads.

PHALARIDEAE

Phalaris canariensis L., Canary Grass; annual, advanced from Europe (pollen mostly perfect).—The chromosomes of this species are rather large and clearly show their tetrad composition. Only six bivalents appear in the easily counted metaphase plates seen in polar view. Cytomyxis is observed in the spireme stages, the mother cells so involved usually disintegrating as a result, as is shown by their occurrence in the latter condition in anthers with normal mother cells. The heterotypic metaphase is quite regular. The fol-

lowing anaphase, however, is often marked by a very tardily splitting chromosome pair. Homeotypic divisions are quite regular, and there is no polycary; nevertheless a small percentage of shriveled pollen may be observed.

Phalaris arundinacea L., Reed Canary Grass; a native lowland meadow species (pollen perfect).—Diakinesis counts show unmistakably seven bivalents, often paired as rings. The size of the chromosomes again brings to light the tetrad structure of the pairs, especially in the heterotypic metaphase preparatory to splitting. Divisions, tetrads, and pollen are in every respect normal.

Phalaris arundinacea L. var. *picta* L., Ribbon Grass; a native of Eurasia and commonly cultivated (pollen 30–40 per cent imperfect). —Diakinesis and heterotypic metaphase plates show fourteen bivalents, the tetraploid complement, one or two bivalents appearing separated as univalents. These univalents consistently lag on the spindle, remain at the poles, or become extruded during the heterotypic metaphase. Not infrequently a bivalent may behave in the same manner. An early anaphase stage shows such laggards still at the poles. The extruded chromosomes seem to disintegrate universally. Laggards at the poles become included in the chromosome groups and subsequently resulting nuclei of the late anaphase. Laggards have been noted in the telophase, but again apparently are always dispersed in the cytoplasm before subsequent divisions are initiated. Homeotypic divisions and tetrads are quite normal in appearance. The phenomenon of cytomyxis is widespread in the species, evidently causing much mother cell disintegration in the spireme stage. Parts of chromosomes may be stranded in adjacent mother cells during the heterotypic metaphase, as a result of cytomyxis in the diakinesis. Chromatin exchange not infrequently occurs again at interkinesis. Cytoplasmic strands without interchanging chromatin may be seen connecting the majority of the mother cells. Polycary has not been observed.

Discussion

Polyploidy

A comparison of the chromosome numbers of the various species and genera of grasses investigated in this research discloses a decided

majority of the cases falling into the groups of the higher multiples of the basic haploid number, which for the most part is seven. The thirteen species reported in this article show three diploids, five tetraploids, four hexaploids, and one octoploid. Grouping these with the species of cereals and allied forms already reported (table I), we find the same correlation in respect to the polyploid tendency. Polyploidy is now known to be of quite general occurrence, especially in the larger and consequently variable families and genera. Its extent is coming to be realized as increasingly widespread in the light of rapidly accumulating data.

The great family Compositae has received attention in several genera. ROSENBERG (**44**) has demonstrated diploids, triploids, and tetraploids in *Hieracium*. The same series with the added category of hexaploids is seen in *Erigeron* (HOLMGREN **23**). *Lactuca* and *Crepis* are complicated by the presence of several series of haploid numbers, but the former reaches a variety which is certainly hexaploid (ISHIKAWA **25**), and the latter attains octoploidy (COLLINS and MANN **9**). A now classic series is shown by the work of TAHARA (**48**) on *Chrysanthemum*, which presents a series running to decaploidy. The *Chrysanthemum* series is far outrivaled in degree of polyploidy by that of *Senecio* (AFZELIUS **2**), which has been shown to have not only decaploids and dodecaploids, but even a species with ninety as the haploid number, the haploid base being five!

The Rosaceae presents further striking examples of the occurrence of polyploidy. TÄCKHOLM'S (**47**) well known work on *Rosa* has demonstrated large polyploid series in all the groups through pentaploidy as well as a few hexaploids and octoploids. A similar exhaustive work on *Rubus* (LONGLEY **32**) has revealed many types falling in all classes of nuclear multiples through hexaploidy. LONGLEY'S work on *Crataegus* (**33**) has shown a considerable amount of triploidy and tetraploidy. *Fragaria* (LONGLEY **35**) contains hexaploids and octoploids.

A brief survey of the occurrence of polyploidy in two large families in addition to the Gramineae has furnished ample evidence of the importance of the phenomena in considering the numbers and diversity of these groups. The cause of polyploidy, especially in the Compositae and the Rosaceae, has been shown by JEFFREY (**27**) to

be due obviously to hybridization, particularly when the concomitant appearance of chromosome misbehavior, polycary and poly-

TABLE I

EXTENT OF POLYPLOIDY IN THE GRAMINEAE

	DIPLOID	TETRAPLOID	HEXAPLOID	OCTOPLOID
Festuca				
ovina*				28
rubra*			21	
duriuscula*			21	
elatior (Evans 13)				
var. arundinacea			21	
var. pratensis	7			
Phragmites				
communis (Tischler 50)		18		
Dactylis				
glomerata* (also Davies 10)		14		
Agropyrum				
repens (Stolze 46)			21	
Triticum (cf. complete list in Gaiser 17)	7	14	21	
Aegilops (cf. complete list in Gaiser 17)	7	14		
Hordeum (cf. complete list in Gaiser 17)	7			
Secale (cf. complete list in Gaiser 17)	7			
Lolium				
perenne (Evans 13)	7			
Avena				
(Gaiser 17)	7	14	21*	
Arrhenatherum				
elatus (Aase and Powers 1)		14		
Ammophila				
breviligulata*		14		
Alopecurus				
pratensis* (also Marchal 39)		14		
geniculatus				
var. aristulatus*	7			
Spartina				
michauxiana*		14		
alterniflora				
var. glabra*			14 bivalents + 14 univalents	
Anthoxanthum				
odoratum (Marchal 39)	8			
Phalaris				
canariensis*	6			
arundinacea*	7			
var. picta*		14		

* Species investigated in this research. Numbers refer to the haploid complement.

spory, are taken into account. The investigators of the several genera previously referred to are wholly in accord with this view. In this connection it is interesting to note how those workers, attack-

ing the problem in *Crepis* from an experimental standpoint, arrived at the same conclusion in regard to the cause of polyploidy. A geneticist's (BABCOCK 3) view is as follows:

> point mutations and chromosome aberrations seem to be so very rare that they could hardly suffice for an explanation of the origin of the thousands of species, genera and families of organisms. Point mutations may start polymorphism, *but hybridization is necessary to produce polyploidy.*

Again, NAWASCHIN (40), after making a detailed study of the individual ground sets of chromosomes in the polyploid series of *Crepis*, concludes that hybridization is the only explanation of the various assortments in different species.

TETRAPLOIDY

In deriving a polyploid series of individuals by the crossing of those with different multiples of chromosomes in their nuclei, the origin of the first multiplication of the normal diploid complement, that is, the tetraploid, is naturally of vital importance. The widespread appearance of tetraploids is everywhere evident. Table I shows the large number of species in this category. DENHAM (12) finds that the Asiatic cottons are normal diploids, but that the New World and Egyptian cottons are tetraploid. Our most common species of blueberries are tetraploid (LONGLEY 36). Many other instances could be cited, such as those in *Rosa* and *Rubus*, to which reference has already been made.

In most experimental cases, tetraploidy is known to be a result of hybridization. The famous "*gigas*" forms of *Oenothera lamarkiana* are clearly hybrids, as shown by their chromosome behavior, and are tetraploids (DAVIS 11). More recently, HÅKANSSON (19) has shown that in hybrids of the cross *O. lamarkiana*×*O. biennis*, both haploid and diploid pollen mother cells may occur in the same anther sac. The case of *Primula kewensis* and that of the tetraploid species of *Datura* are other well known examples of a doubling of diploid complement arising as a result of crossing. The most recent and convincing work in this connection is that of KARPECHENKO (29). In a cross between species of *Raphanus* and *Brassica*, the F_1 generation gives rise to some plants that produce gametes with the somatic complex of chromosomes (doubling having taken place from a failure

of the mates to pair and initiate the reduction division). The F_2 generations of such types are tetraploid and do not revert. KARPECHENKO states:

> Having like the F_1 hybrids a pod of very peculiar structure, which characterizes them as distinct species, the tetraploid F_2 hybrids acquire quite regular reduction divisions, full fertility, and moreover, prove unable to cross with one of their parents—*Brassica*. It seems that we here approach nearer than we ever did the experimental reproduction of one of the processes in species formation.

In connection with the consideration of the doubling of a given chromosome complement, there has been much speculation to show that the doubled number represents merely a duplication of the fundamental set. Of course, such a chromosome doubling takes place in cases of apogamous reproduction, but there is no evidence to show how tetraploid or polyploid individuals arise in consequence. SAX (**45**) does not see how differential chromosome matings involving unpaired members or univalents can occur in *Triticum* hybrids if polyploidy is the result of a mere duplication of the original set.

The theory of the origin of tetraploidy that thus far seems most plausible in the light of available evidence is that of the "indirect chromosome union" put forward by WINGE (**53**). It is conceivable that in a given cross there will be no pairing of chromosomes derived from the parent gametes at the time of the reduction division. Yet,

> if the chromosomes are to find a partner, then each of the chromosomes in the zygote must divide, for thus indirectly to produce a union of chromosomes and we must assume that this is realized in the hybrid zygotes that have any possibility at all of propagating. The hybrid sporophyte thus produced will then have $4x$ chromosomes.

WINGE'S theory will be seen to differ from other theories of chromosome doubling in that it brings into play the act of hybridization, the resultant chromosome incompatibility producing the duplication of haploid sets which on segregation give rise to a new qualitative as well as quantitative assortment of chromosomes in the offspring. This theory finds strong support in the work of KARPECHENKO just mentioned.

LAGGING UNIVALENTS

Having considered quantitative differences in the chromosome equipments, namely, polyploidy as a criterion of hybridization, the

equally important qualitative differences as ear marks of the same situation may now be discussed. The presence of unpaired or univalent chromosomes is one of the most striking indications of a hybrid. Such chromosomes are well instanced in the case of *Spartina alterniflora* var. *glabra*.

The classic example of lagging univalents is ROSENBERG'S (**43**) *Drosera* hybrid, in which ten univalents lag on the spindle and follow a random distribution to the poles in both divisions. Some univalents may be extruded, resulting in polycary and polyspory. Experimentally produced hybrids often show similar chromosome behavior, particularly in triploids resulting from crosses of tetraploids with diploids. Among these may be cited *Papaver somniferum*×*P. orientale* (YASUI **54**), and *Nicotiana sylvestris*×*N. tabacum* var. *purpurea* (GOODSPEED and CLAUSEN **18**), the first species being the diploid in each instance. A very striking case in nature is seen in a hybrid *Isoetes* (JEFFREY and HICKS **28**). Analogous cases also occur naturally in *Senecio* (AFZELIUS **2**) and *Hieracium* (ROSENBERG **44**).

In *Spartina*, as well as *Papaver* and *Hieracium*, some of the univalents appear to split in the heterotypic division. In *Viola* crosses CLAUSEN (**6**) reports conditions from a splitting of part of the univalents to instances where they all divide in the first division after the bivalents have passed to the poles. This latter situation is typical in triploid *Triticum* hybrids (THOMPSON **49**).

NON-PAIRING

Several species of grasses in this research present another category of univalent chromosomes in which the numerical complement of the nucleus furnished each chromosome with a mate, but a few do not pair because of some lessened affinity existing between them. It has been noted in *Alopecurus pratensis*, for example, that one or two sets of chromosomes are seen unpaired in the diakinesis, and, further, that these may lag on the heterotypic spindle in this condition, at times being extruded into the cytoplasm. *Dactylis glomerata* presents a similar situation, in which the late diakinesis may be found with all the chromosomes unpaired, not a few of these lagging as univalents on the spindle. *Phalaris arundinacea* var. *picta* consistently displays unpaired mates as laggards and extrusions.

Phenomena analogous with the foregoing have been reported in certain varietal forms of *Oryza sativa* (KUWADA **30**). DAVIS (**11**) noticed an unpaired chromosome set consistently appearing in the diakinesis of *Oenothera biennis*. The same condition has been shown since to be common in several other species of this genus, famous for its hybrid members (CLELAND **7**). Some of the diploid species of *Rosa* whose ancestry might be questioned as to purity also display unpaired chromosomes in the early stages of the maturation divisions.

LAGGING BIVALENTS

A lack of uniformity in the movements of chromosomes during the maturation division is not dependent on the presence of univalents, however. Many of the species examined in this study showed bivalent laggards. The three species of *Festuca*, and *Avena sativa*, *Ammophila breviligulata*, and *Alopecurus pratensis*, all display lagging bivalents at both metaphase and anaphase of the heterotypic division. Lagging and extrusions paralleling these instances may be seen in many of the cases cited in the discussion of polyploidy, such as *Rosa*, *Rubus*, *Crataegus*, *Fragaria*, *Primula*, and *Gossypium*. The same meiotic aberrations have recently been correlated with polycary and polyspory in the pentaploid and hexaploid species of *Vaccinium* (LONGLEY **36**) and in the several species and varieties of *Aesculus* (HOAR **22**).

UNEQUAL DISTRIBUTION

Extreme conditions of lagging bivalents often may be operative in effecting an unequal segregation of chromosomes to the poles in the heterotypic anaphase. More specifically, one or more lagging bivalents remain at or near one of the poles during the metaphase, and if not extruded, will be included in the subsequently formed nuclei. Striking instances of this phenomenon have been observed in *Alopecurus geniculatus* var. *aristulatus*, *Avena sativa*, and *Phalaris arundinacea* var. *picta*. Conditions similar to these are instanced in several varietal forms of *Citrus* (FROST **16**), and in *Potentilla anserina* var. *grandis* (ROSCOE **42**), where the situation is complicated still further by the presence of univalents.

Pollen viability

The common result of extruded chromatin from irregular divisions in hybrids such as have been cited is the appearance of polycary and polyspory and consequently sterile pollen. It is noteworthy that in the grasses investigated polyspory has not been observed, yet polycary and sterile pollen are frequent in appearance. A striking parallel situation is seen in wheat hybrids, where polyspory is absent in the presence of polycary, and 98–100 per cent of the pollen is sterile (Thompson **49**).

The case of *Avena sativa* displays the unusual condition of apparently perfect pollen in the presence of a considerable amount of polycary. The so-called "fatuoid mutants" of this obviously hybrid species show the same degree of viable pollen production (Huskins **24**). Of course known hybrids often have perfectly good pollen, yet a considerable amount of work on wheat hybrids (Thompson **49**) has demonstrated that a high percentage of the apparently good pollen will not germinate, and, further, that in the case of seeds derived by the use of such pollen as will germinate, 50 per cent of the offspring never mature.

Cytomyxis

In a discussion of the nuclear phenomena of plant hybrids, we have thus far noted and considered polyploidy and irregularities in chromosome behavior with the resultant degenerate condition of the germ cells. There is still another apparent abnormality which has been observed extensively in the pollen mother cells of the grasses in this study, and that is cytomyxis. More convincing evidence as to the occurrence of cytomyxis in connection with hybrids is offered by an investigation of the grasses in the tribe Panicae, and for this reason the discussion of the phenomenon will be left for a later article.

Conclusions

Festuceae

Festuca.—The relatively high degree of polyploidy in the species studied (*F. ovina* twenty-eight, and *F. rubra* and *F. duriuscula* both twenty-one) is quite consistent with the size of the genus. There are about one hundred species known, forty of which are in the United

States. The varieties of *F. ovina* (TURESSON 51) and *F. rubra* are legion, and they are reported to cross with one another freely (COCKAYNE in LOTSY 37). Taxonomic characters designed to distinguish these two common species are not at all clearly demonstrable in many instances. The presence of lagging chromosomes, polycary, and sterile pollen witnesses the hybrid origin of all three of the particular strains of the species investigated.

Dactylis glomerata.—Hybrid criteria are not outstanding in this species, yet tetraploidy, sterile pollen, lessened chromosome affinity, and the presence of two other species in the genus (including many European varieties, HEGI 20) taken as a whole make the hybrid origin of this species seem quite probable.

AVENEAE

Avena sativa.—This is a rather large genus of about fifty-five known species. The hybrid origin of many cultivated forms of this species has already been indicated by the work of HUSKINS (24). This escaped variety shows lagging and polycary to an even greater degree than in those heretofore reported.

AGROSTIDEAE

Ammophila breviligulata.—As in *Dactylis*, this is a small genus but with a somewhat wide dispersal, the two European species being distinct from the North American ones. Lagging chromosomes and a large amount of sterile pollen as well as tetraploidy bear witness to a hybrid origin of this species, however remote its occurrence.

Alopecurus.—Both *A. pratensis* and *A. geniculatus* may run to varieties (HEGI 20) in this genus of twenty-five species, eight of which are found in the United States. Cytological evidence of hybridity, mostly in the form of lagging chromosomes and sterile pollen, is abundant in the diploid variety of *A. geniculatus*, and only less marked in the tetraploid *A. pratensis*.

CHLORIDEAE

Spartina.—This is a rather extensive genus of fourteen species, mostly in North America. The hexaploid *S. alterniflora* var. *glabra* with its fourteen univalent chromosomes is undoubtedly a hybrid.

Cytological evidence of crossing is rather meager in *S. michauxiana;* still an appreciable amount of bad pollen together with tetraploidy suggests hybridity.

PHALARIDEAE

Phalaris.—There are two distinct taxonomic sections in this genus of about twenty species in Europe and the United States. *P. canariensis*, of the section Euphalaris, does not bear marked evidence of a hybrid origin, other than a small percentage of sterile pollen and a possible aberration from the normal seven basis in the haploid count of six. In the section Digraphis, *P. arundinacea* is undoubtedly a pure, diploid species. This species may run to some five varieties in Europe (HEGI 20), and the cultivated tetraploid variety *picta* clearly shows its hybrid origin by the presence of lagging chromosomes and an appreciable amount of sterile pollen.

Summary

1. The following members of the tribes Festuceae, Aveneae, Agrostideae, Chlorideae, and Phalarideae have been investigated.

Diploids: *Alopecurus geniculatus* var. *aristulatus*, *Phalaris canariensis P. arundinacea.*

Tetraploids: *Dactylis glomerata*, *Ammophila breviligulata*, *Alopecurus pratensis*, *Spartina michauxiana*, *Phalaris arundinacea* var. *picta.*

Hexaploids: *Festuca rubra*, *F. duriuscula*, *Avena sativa* var. *mutica*, *Spartina alterniflora* var. *glabra.*

Octoploid: *Festuca ovina.*

2. Lagging univalents are found in *Spartina alterniflora* var. *glabra.*

3. *Phalaris arundinacea* is considered a pure, diploid species.

4. Varying degrees and combinations of non-pairing, lagging and extrusion, cytomyxis, polycary, and sterile pollen are found in all other species.

5. No cases of polyspory are noted.

6. Polyploidy or cytological abnormalities of the maturation divisions or both are considered as evidence of the hybrid origin of all species except *Phalaris arundinacea.*

This research has been carried out at the suggestion of Professor E. C. JEFFREY, to whom I am greatly indebted for advice in its prosecution.

LABORATORIES OF PLANT MORPHOLOGY
HARVARD UNIVERSITY

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EXPLANATION OF PLATES XXV–XXVII

PLATE XXV

× 2300 diameters

Festuca duriuscula L.

FIG. 1.—Heterotypic metaphase showing lagging and extruded bivalent.

FIG. 2.—Heterotypic anaphase with lagging univalents and extruded bivalents.

FIG. 3.—Interkinesis; diad split nearly completed; chromatin connections between nuclei of adjacent mother cells.

FIG. 4.—Diad stage practically completed; chromatin in path of obliterated spindle and excluded from the nuclei.

FIG. 5.—Homeotypic divisions; no irregularities.

Festuca ovina L.

FIG. 6.—Heterotypic metaphase; laggards and extrusions.

FIG. 7.—Late heterotypic anaphase; lagging bivalents at equator.

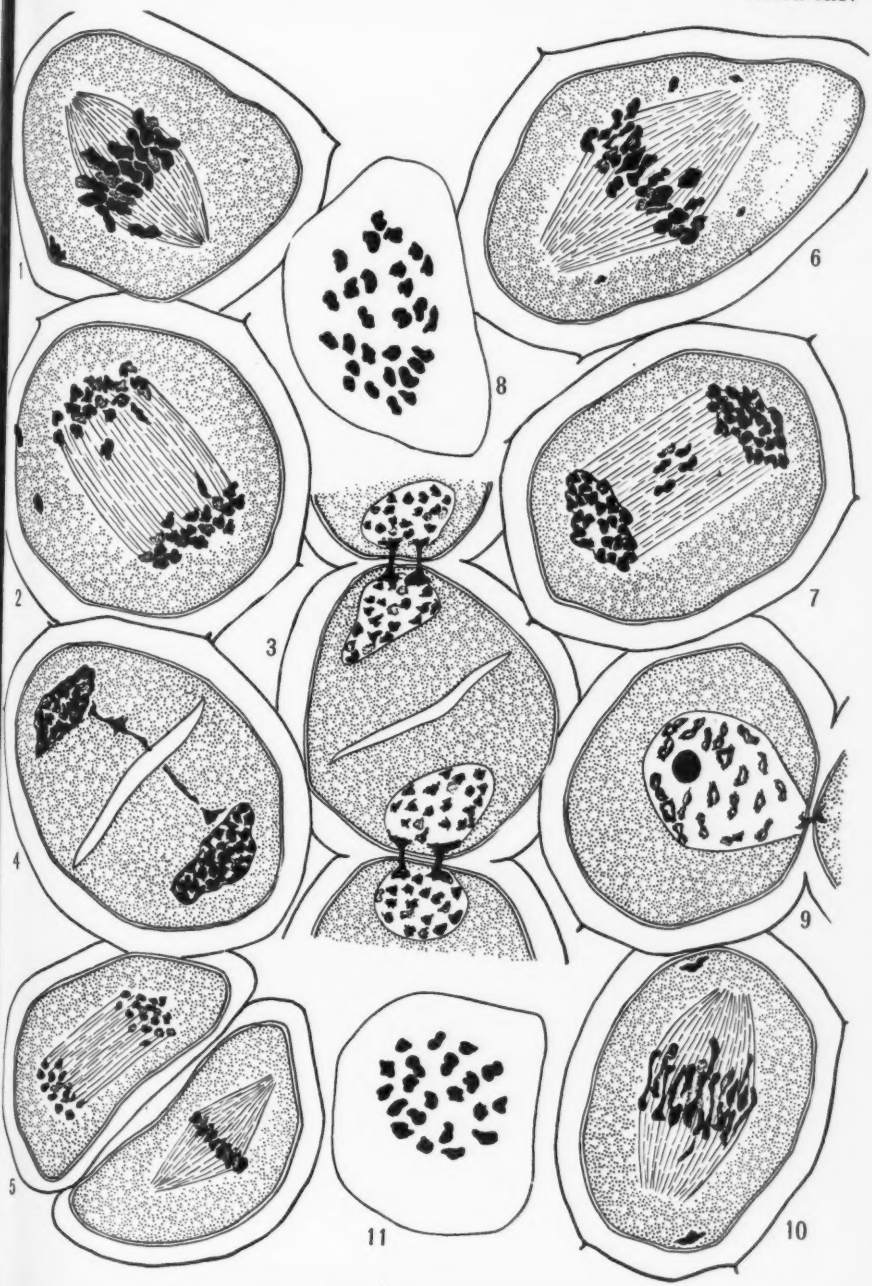
FIG. 8.—Heterotypic metaphase, polar view, showing twenty-eight bivalents at plate.

Festuca rubra L.

FIG. 9.—Diakinesis, twenty-one pairs, one partly stranded in adjacent mother cell.

FIG. 10.—Heterotypic metaphase with extruded chromosomes.

FIG. 11.—Heterotypic metaphase, polar view, showing twenty-one bivalents.



CHURCH on GRAMINEAE

12

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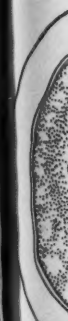

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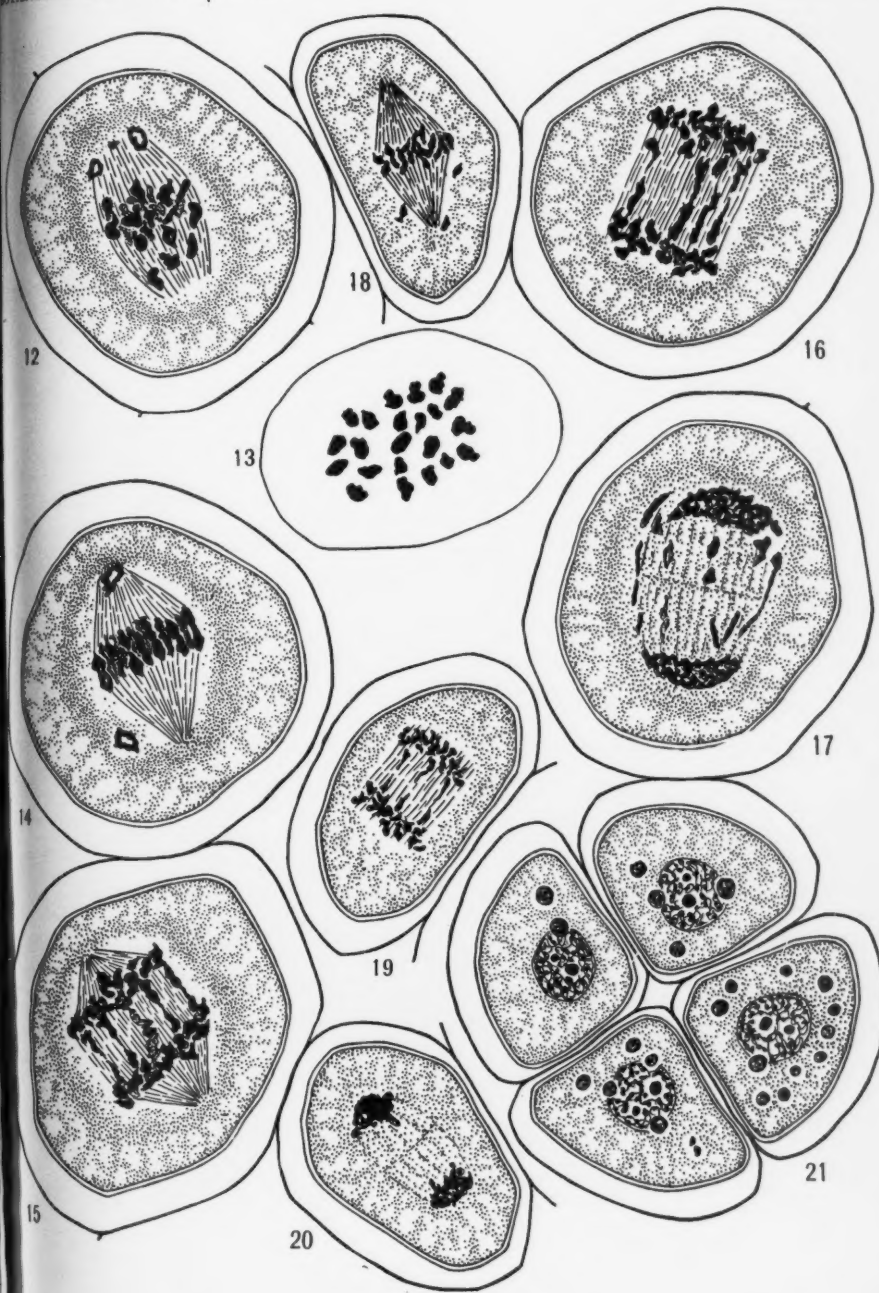
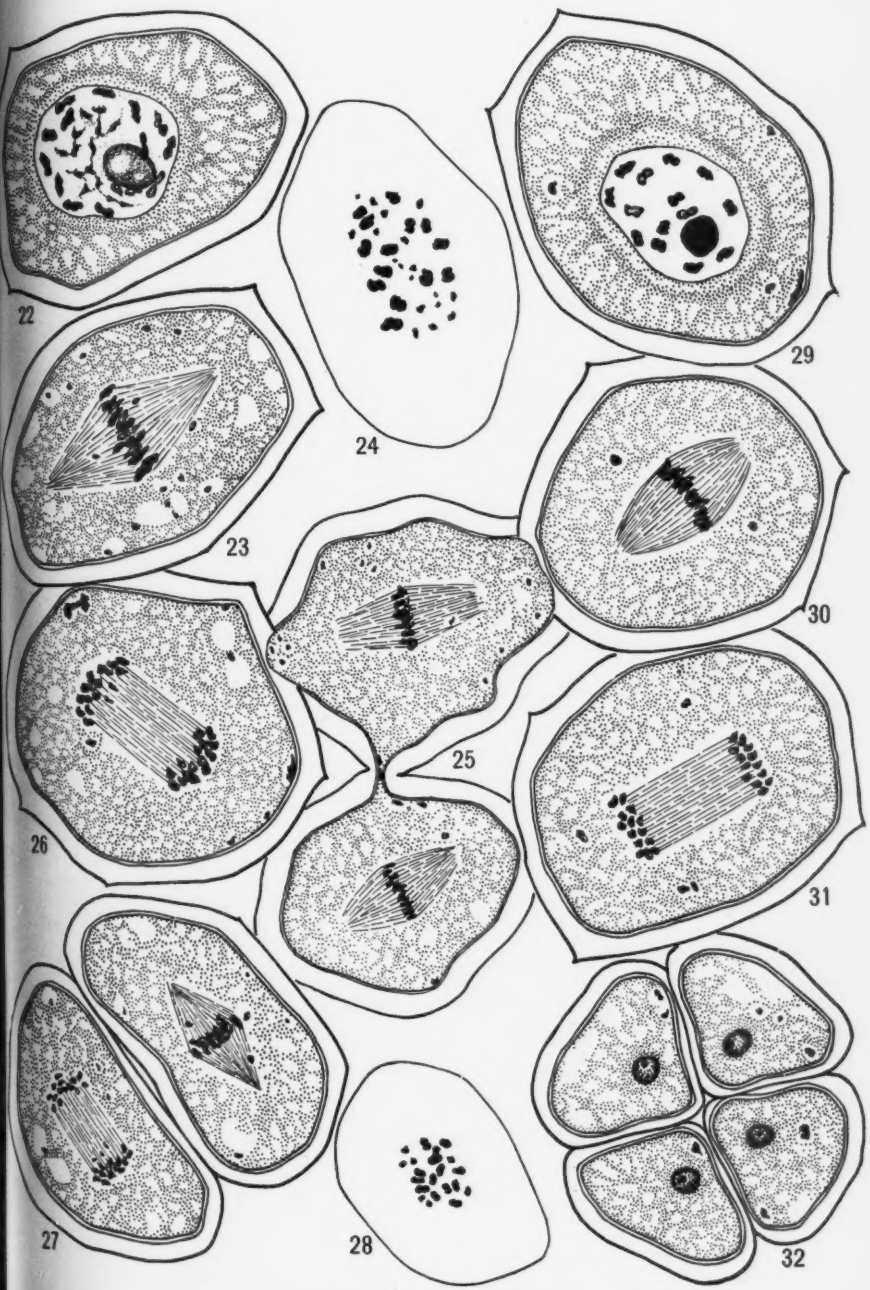


CHURCH on GRAMINEAE

OTANICA
22
26



CHURCH on GRAMINEAE

PLATE XXVI

× 1900 diameters

Avena sativa L.

FIG. 12.—Early heterotypic metaphase; laggards, especially ringed bivalents at poles.

FIG. 13.—Heterotypic metaphase; polar view showing twenty-one bivalents.

FIG. 14.—Late heterotypic metaphase; note lagging and extruded bivalent.

FIG. 15.—Early heterotypic metaphase; five pairs of chromosomes late in splitting.

FIG. 16.—Late heterotypic anaphase still showing laggards.

FIG. 17.—Heterotypic telophase; laggards excluded from nuclei.

FIG. 18.—Homeotypic metaphase; note extrusions.

FIG. 19.—Homeotypic anaphase showing laggards.

FIG. 20.—Homeotypic telophase; some chromosomes barely reached nuclei.

FIG. 21.—Tetrad showing considerable polycary.

PLATE XXVII

× 2300 diameters

Spartina alterniflora Loisel var. *glabra* (Muhl.) Fern.

FIG. 22.—Diakinesis showing fourteen bivalents and fourteen univalents in center of nucleus.

FIG. 23.—Heterotypic metaphase; note many extruded univalents.

FIG. 24.—Heterotypic metaphase, polar view, showing fourteen bivalents, eleven univalents, and three split univalents.

FIG. 25.—Two mother cells showing cytomyxis ×1540.

FIG. 26.—Heterotypic anaphase; note coalescing and extruded univalents.

FIG. 27.—Homeotypic divisions; univalents still presenting irregularities.

FIG. 28.—Homeotypic metaphase, polar view, showing univalents and bivalents at plate.

Spartina michauxiana Hitch.

FIG. 29.—Diakinesis, showing fourteen bivalents; note chromatin in cytoplasm.

FIG. 30.—Heterotypic metaphase, presenting regularity except for persistent cytoplasmic chromatin.

FIG. 31.—Heterotypic anaphase; again regularity except extrusions.

FIG. 32.—Tetrad showing polycary and vacuolated condition of constituents.

MOTTLE-LEAF IN CITRUS ARTIFICIALLY PRODUCED BY LITHIUM[1]

A. R. C. HAAS

(WITH FOUR FIGURES)

The mottle-leaf problem is an intricate one not readily understood, owing to the fact that artificially in controlled cultures it has been difficult to produce the mottling satisfactorily over a period of time by means of chemically pure salts, and to the fact that it has been found difficult to improve trees badly affected with mottle-leaf in the field. KELLEY and CUMMINS (6) have found the composition of mottled citrus leaves from the field to contain larger amounts of potassium, phosphorus, and frequently of nitrogen, but smaller amounts of calcium than normal leaves. The composition of mottled citrus leaves resembles that of immature citrus leaves.

The writer has attacked the mottle-leaf problem in three ways: (1) Poisoning the leaves so as to keep them from becoming mature from the standpoint of composition; (2) replacement of the calcium of the soil with different bases and leaching out of much of the liberated calcium; (3) locking up much of the supply of soluble calcium in the soil and possibly within the plant by the use of alkaline substances without leaching out the calcium from the soil.

In a recent paper, HAAS, BATCHELOR, and THOMAS (3) have pointed out in connection with yellows or little-leaf of walnut trees that the absorption of a toxic agent in sufficient concentration may be an important factor in producing such diseases. In the present paper the writer will report experiments dealing with the first method of approach, and will deal with the effects of lithium as a toxic agent in the artificial production of mottle-leaf of citrus.

So far as the writer is aware, no report has been made of lithium as a constituent of mottled citrus leaves in the field. Whether or not upon investigation of mottled leaves in the field lithium is found to be present, the data nevertheless are of scientific value in elucidating the nature of the physiological disease called mottle-leaf.

[1] Paper no. 194, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

Lithium has the lowest atomic weight of the non-gaseous elements in Group I in the periodic system, and has long been used in physiological laboratories in studies involving ascent of sap, etc., because of its ease of movement and qualitative determination. The writer has also concluded experiments in which other elements of low atomic weight have been employed, and has succeeded in artificially producing mottle-leaf of walnut trees in the field, but these results must be presented at a later time.

When a concentration of lithium equal to 0.2 parts per million was used in conjunction with similar amounts of B, Al, I, Ti, Br, Sr, Mn, and NH_4 (HAAS and REED 4) in the culture solution for the growth of citrus in sand, the trees grew in a normal manner. In order to obtain evidence in regard to the rôle of each constituent of this "A-Z" solution that was used in overcoming the inorganic deficiencies of Hoagland's solution (REED and HAAS 7) for the growth of citrus, sand cultures were conducted in galvanized iron containers 18 inches diameter $\times$26 inches deep. Orange and lemon trees budded on sour-orange stock were planted in these containers, and a single but different constituent was omitted from the "A-Z" solution in each series. The strength of the modified "A-Z" solutions that were used in Hoagland's solution in these cultures was five times that of the "A-Z" solution originally used. The first application of these culture solutions was made at the time of planting the trees, so that the new roots were subject to the treatment from the very beginning of growth. Comparison was possible with many other sand cultures of citrus growing at the same time.

One Valencia orange and two Eureka lemon trees comprised each series. After one year's growth, the leaves of the lemon trees, in all but one series, began to wilt and absciss, beginning with the oldest leaves; while the orange trees maintained excellent growth. Fig. 1 shows the vigorous growth of the orange trees (left) in each series, and the permanently wilted condition of the leaves of the lemon trees (center and right) in each series except one. The only series in which the lemon trees made good growth was that in which lithium had been omitted ("A-Y") from the "A-Z" solution. The wilted condition of the leaves of the lemon trees that had received a culture solution containing 1 p.p.m. of lithium was very striking,

and was not related to the supply of moisture in the sand. Upon removal of the trees from the sand, it was found that the roots had become soft and gelatinous. The results suggested that the lithium was toxic and that Valencia orange trees were less sensitive than Eureka lemon trees to this toxic agent. The toxicity of this small concentration of lithium was the greater owing to the fact that the

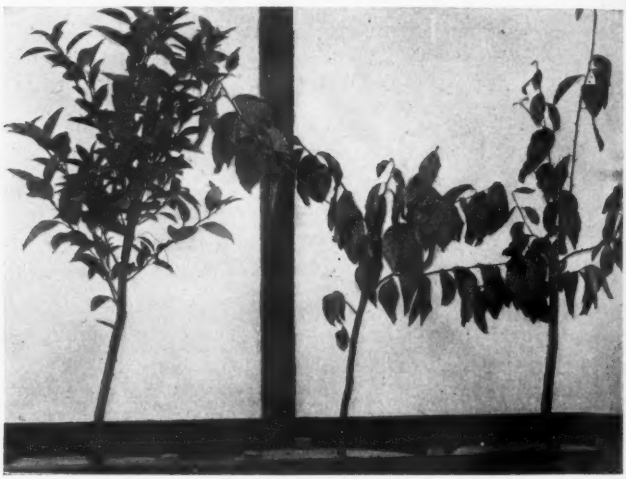


FIG. 1.—Left, Valencia orange tree typical of sand cultures receiving Hoagland's culture solution containing 1 p.p.m. of certain elements not ordinarily added to culture solutions (five times "A–Z" concentration) or lacking any one of elements of "A–Z" solution; center and right, Eureka lemon trees permanently wilted in sand culture when culture solution contained 1 p.p.m. of lithium regardless of other elements of "A–Z."

culture solution containing lithium was applied from the beginning of growth, when the pruned tree consisted of only the lower two-thirds of the bare trunk and a clean main root about a foot in length.

In another experiment with citrus tree cultures in containers such as were previously described, budded Eureka lemon trees were given Hoagland's culture solution and were allowed to grow for several months, after which 25 p.p.m. of lithium was added to the culture solution. Many of the new leaves began to mottle as they became older, and showed dried areas along the leaf margins as

well as in the mottled portion, as shown in fig. 2. As a rule, leaves that were already mature did not mottle but became dry along the leaf margin and between the veins. As the drying of the leaves became severe, many of them abscissed. The new leaves were much wrinkled, and were pale yellowish green except near the midrib, where they were of a darker green (fig. 3). Upon removal of the lithium-containing culture solution from the sand and its replacement with a similar solution lacking lithium, new growth developed

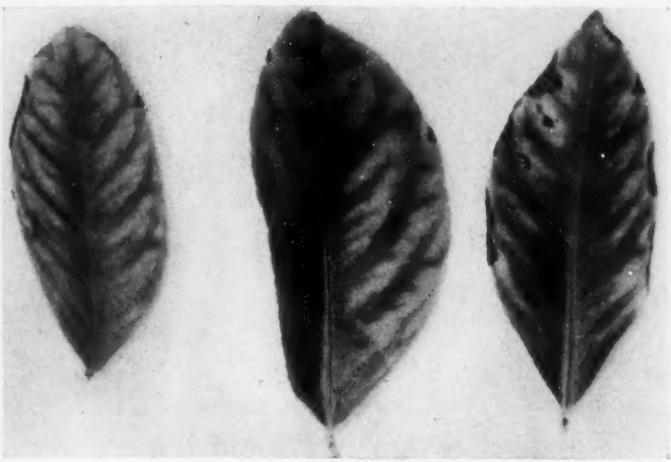


FIG. 2.—Toxic effects of lithium on lemon trees in sand culture

and the leaves of the successive cycles of growth were less and less wrinkled, until finally normal growth was produced.

No effects of lithium upon the external appearance of the citrus roots have been observed, and upon removal of the lithium from the solution bathing the roots, the root growth is resumed provided other environmental conditions are favorable. It appears that, with a toxic agent such as lithium, which is not readily precipitated within the conducting system of the tree, after equilibrium is established within the tree the toxic agent concentrates in the leaves as a result of transpiration. Consequently upon removal of the toxic agent from the solution bathing the roots, the dilution of the toxic agent

takes place most rapidly in the roots, with the result that the root growth then made is most important to the recovery of the tree. The plant evidently gets rid of the lithium contained within its conducting tissues largely through the production of new growth, rather than by concentrating all of the toxic agent in the already injured leaves.

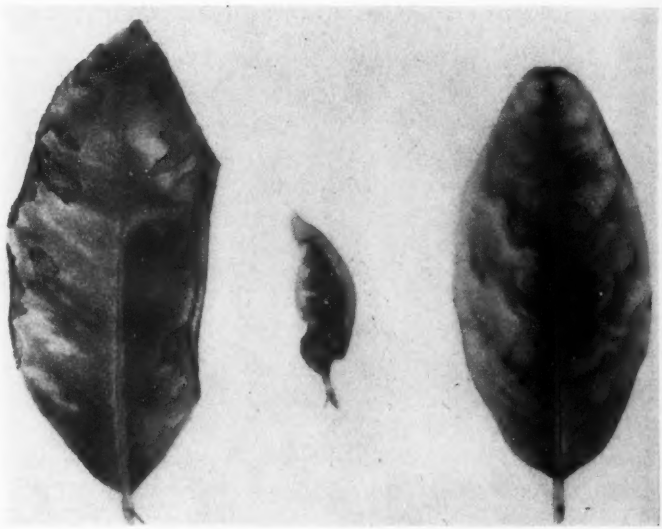


FIG. 3.—Toxic effects of lithium on lemon leaves in sand cultures; left, mature leaf burned between veins and along margin; center and right, chlorotic, crinkly, and thin new leaves.

The assumption is made that the lithium accumulations between the veins and along the margins of the veins are unequal in amount, and affect growth to different degrees as a result of such unequal depositions. As a consequence the leaves become badly crinkled, regardless of their size. FAWCETT and LEE (2) have described a crinkly leaf condition of lemon trees in California, and point out the possible relationship between this condition and insect effects, as well as the possible hereditary transmission of such abnormalities. The crinkly leaf condition seen in fig. 3 has been produced by a

known cause, and the results add to our knowledge of the causation of observed physiological effects. Additional experiments have also shown that a crinkly leaf condition of lemon leaves may be produced by large concentrations of nitrate in the solution bathing the roots. In this case it is reasonable to assume rapid but unequal rates of growth of tissues, with different capacities to utilize the abundant nitrate.

The effect of lithium on citrus trees was then studied in soil cultures in 10 gal. earthenware jars, the soil used being that classified as Sierra loam, which was obtained near the Citrus Experiment Station. The leaves and shoots of the budded Valencia orange trees were pruned off, but the washed root system was left intact when the trees were planted in the jars. The cultures were grown in the glasshouse from April until the following March. Immediately after the trees were planted in the jars, the soil in each jar received 2 liters of Hoagland's culture solution containing respectively 0, 3, 6, 9, 12, and 15 parts of lithium per million parts of dry soil, followed by 2 liters of a similar solution in which lithium was absent. Although the culture jars had drainage outlets, no drainage water was allowed to escape. In case of drainage, the solution was returned to the jar from which it came. The experiment was begun in April, and by the following February all of the trees had died except the control trees and the one in soil containing 3 p.p.m. of lithium. The control trees were normal in appearance, while the tree in the culture containing 3 p.p.m. of lithium had many burned leaves which fell prematurely. Some leaves were small, and their appearance resembled that of mottled leaves. Every leaf of the trees in cultures that received 6 p.p.m. or more of lithium, burned and was shed prematurely. The succeeding leaves fared no better than their predecessors. Finally all of the shoots died back to the trunk.

The toxicity of lithium was severe, even though only one application of solution containing lithium was made during the 11-month experimental period. Samples of burned leaves were obtained by shaking the tree trunks and catching the falling leaves before they touched the soil. Analyses of these leaves by Mr. S. M. BROWN, Assistant Chemist in the Agricultural Chemistry Division of this Station, showed that the amount of lithium in the burned leaves was

too small to be detected gravimetrically, but that spectrum analysis gave positive tests for its presence in the affected leaves.

Water cultures in quart Mason jars were begun on April 19 with five sweet orange seedlings per jar and three jars in each series. Hoagland's nutrient solution was used, and lithium additions were made so as to have concentrations from 0 to 7 p.p.m. of lithium in the culture solution. On the following June 1 there was slight marginal discoloration and burning in the 2 and 3 p.p.m. concentrations, with severe burning at the higher concentrations. Similar water cultures were conducted with lemon seedlings from April 27 until the following June 20. At 1 p.p.m. there was a slight burning at the tip and along the leaf margin, and the new leaves were very chlorotic. At higher concentrations these effects were more severe, with abscission of most of the leaves and ultimate death of the seedlings.

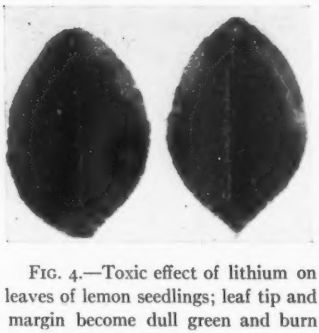


FIG. 4.—Toxic effect of lithium on leaves of lemon seedlings; leaf tip and margin become dull green and burn very rapidly after discoloration; abscission of burned leaves most rapid.

A field experiment was begun in October, 1926, at the Rubidoux tract of the Citrus Experiment Station, in which lithium nitrate was applied to the trees in basins. A row of Smith's Early navel orange trees on sweet-orange rootstock (C. E. S. no. 46) were selected as being uniformly good trees, relatively free from mottle-leaf throughout the entire row. The trees were approximately 20 years old. The upper three trees in the row were irrigated by means of furrows, and the remainder were irrigated by the quartered basin method. The first or uppermost basined tree, together with the three furrow-irrigated trees, served as controls. The other basined trees, beginning with the tree below the controls, received respectively lithium nitrate ($LiNO_33H_2O$) in the following amounts: 25, 50, 75, 100, 150, and 200 gm. Applications of these amounts were made on the following dates: 10/15/26, 2/2/27, 6/20/27, 7/23/27, 8/30/27, 10/21/27, 3/12/28, and 5/1/28, eight applications in all. In a single applica-

tion the lithium applied was 1.41, 2.82, 4.23, 5.64, 8.46, and 11.28 gm. respectively. Eight applications, therefore, have given the basins 11.28, 22.56, 33.84, 45.12, 67.68, and 90.24 gm. of actual lithium respectively during the course of the experiment. The trees have gradually been losing many of their leaves at the higher concentrations, and the remaining leaves are badly mottled. At the lowest concentration of lithium there is only a small amount of mottle-leaf, the tree being not appreciably affected as yet. At the second lowest concentration many of the leaves are curled from the apical end toward the ventral side. Many of the leaves are undersized, willow-like in outline, and severely mottled. Above this concentration the greatest apparent change is in the increasing loss of leaves. Some of the leaves on the trees that received the larger concentrations were burned near the tip prior to abscission.

In order to get a better understanding of the nutrition of normal and mottled leaves, studies were made on sap obtained by means of air suction upon comparable portions of normal and mottled citrus trees. The method employed was essentially that described by BENNETT, ANDERSSEN and MILAD (1), the sap being withdrawn by means of a battery of suction flasks as the plant material was cut shorter and shorter. The material consisted of the trunks of twelve normal Valencia orange trees 2 to 3 years of age, and of two sets of twelve each of badly mottled Valencia orange trees also 2 to 3 years of age; all trees having been budded on sour-orange stocks. Sap was also withdrawn from the twelve roots of the normal trees and from the lot of twenty-four roots of the mottled trees. Twenty-four branches, $1\frac{1}{4}$–$1\frac{1}{2}$ inches in diameter at the larger end, were taken from normal and an equal number from badly mottled Washington navel orange trees budded on sweet-orange stocks, at the Rubidoux tract of the Citrus Experiment Station, and their sap extracted by the method described. The sap obtained in each case was ashed and a partial analysis was made for sodium, potassium, calcium, and magnesium. The results obtained are given in table I, where it is seen that the sap of the trunks of mottled Valencia orange trees and of the branches of mottled Washington navel orange trees was not lower in calcium content than the sap of comparable portions of normal

trees, although a smaller amount of calcium was found in the sap of the roots of the mottled than in that of the roots of the normal trees. Sap analyses are as yet difficult of interpretation, because we do not know how rapidly the bases found in the tracheal sap are renewed by root absorption when they are removed from the sap by the leaves or other plant organs.

The absorbed lithium is assumed to act as a poison to the leaves, preventing them from changing their ash composition from that characterizing immature leaves to that of mature leaves. In this

TABLE I

COMPOSITION OF SAP OBTAINED BY SUCTION FROM COMPARABLE PORTIONS OF NORMAL AND MOTTLED CITRUS TREES (PARTS PER MILLION)

	TRUNKS OF VALENCIA ORANGE TREES, 2–3 YEARS OLD, BOX SPRINGS TRACT, CITRUS EXP. STA., MARCH 24, 1927			ROOTS OF VALENCIA ORANGE TREES, 2–3 YEARS OLD, BOX SPRINGS TRACT, CITRUS EXP. STA., MARCH 24, 1927		BRANCHES (1½–2½ INCHES DIAM.) OF 21-YEAR-OLD NAVEL ORANGE TREES, RUBIDOUX TRACT, CITRUS EXP. STA., APRIL 7, 1927	
	Normal	Badly mottled	Badly mottled	Normal	Badly mottled	Normal	Badly mottled
Ash	546	347	449	914	738	452	665
Na	287	252	303	.....	482	66	52
K	230	145	174	.....	253	91	151
Ca	81	105	119	201	155	80	126
Mg	38	35	40	52	68	.....	.....
pH	.....	.....	.....	.....	.....	6.2	6.0

case it is assumed that the toxic substance prevents the leaves from obtaining the requisite amount of calcium, etc., for the normal functioning of chlorophyll. If this is true, then the affected leaves should be unable to utilize the available calcium, etc., even though an abundance be at hand, somewhat as in the case of cyanide poisoning in which the individual breathes heavily to get an abundance of oxygen but the system cannot utilize it and death occurs from asphyxiation. We may consider two possibilities as the modus operandi. (1) The toxin may be supposed to act directly on the chlorophyll to break it down and bring about the mottling, the inorganic constitution being only that occurring in the leaves at the time the mottling develops. In this case mature leaves having the full complement of calcium, etc., should be found to mottle, which is not the case. If they show injury at all they do not mottle but burn and

absciss. This fact might be explained on the supposition that the mottling stage has been passed by so rapidly as to obscure its appearance, but this hypothesis is not tenable on account of the fact that in many such cases the burning is at the tip rather than between the veins. Burning between the veins is often preceded by mottling, although the mottling may be of short duration. Mature leaves are not known to mottle if normal up to the time of becoming mature with respect to their inorganic composition. (2) It may be assumed that it is the inorganic constitution of the leaves brought about by the toxin that determines whether or not the leaves mottle. This hypothesis is supported by the fact that in unpublished data on the growth of citrus cuttings in water cultures and in other types of cultures, mottling has been brought about by certain modifications of the inorganic nutrition of the plants. Furthermore, when desiccating winds defoliate normal orange trees, frequently some of the succeeding growth may be considerably mottled. This has been attributed by HAAS and REED (5) to losses of inorganic constituents from the portions of the trees that normally furnish new leaves with requisite amounts at the proper rate. The second view does not explain why some normal appearing leaves occur on badly mottled trees and yet have the composition of immature or mottled leaves. This fact was long a serious obstacle in the interpretation of analytical data from affected material for which the normal or control portions were obtained from the affected trees rather than from entirely normal trees. Possibly the chlorophyll in some leaves may be able to resist a wider range in inorganic constitution than that of other leaves on the same tree, or perhaps these normal appearing leaves are soon to mottle.

The former view assumes a direct effect of the toxin on the chlorophyll molecule; the other view supposes an indirect action upon the processes of chlorophyll formation and decomposition through the inorganic constitution of the protoplasm of the leaf tissues.

It is not to be concluded that all leaf poisons will produce the same type of effect upon the chlorophyll of the leaves. It is definitely known from unpublished results that the effects of different poisons may be slightly different from one another, and that there are various types of mottle-leaf. In each case the inorganic composi-

tion of the mottled leaves is typical of that found in immature normal leaves.

Different toxic substances may move to different locations in the leaf: some toxins may affect the leaf near the apical region, and if in sufficient concentration may also affect the basal region; whereas, other toxins may act between the veins over the entire leaf area from the beginning of visible injury.

There is no a priori reason why certain organic substances may not be found that may affect citrus leaves in the manner that lithium does. This seems probable in view of the fact that frequently on certain soils continued use of certain organic fertilizers has been accompanied by severe cases of mottle-leaf. Although much work has been done with inorganic toxic agents other than lithium, the inclusion of many more organic and inorganic toxic substances in such studies may help to clarify our knowledge of the nutrition of trees under irrigated agriculture.

Summary

1. Mottle-leaf of citrus has been produced artificially in sand and soil cultures as well as in the field by the use of lithium. The lithium is assumed to act as a poison to the growth processes going on within the leaves, so that they are unable to utilize fully the inorganic salts in the tracheal sap, and as a consequence of the effect of the poison on the growth processes the old mottled leaves have the composition of immature normal leaves.

2. Analyses of the sap of comparable portions of mottled and normal citrus trees in the field have shown that, although the leaves of mottled trees may show a deficiency of calcium, the tracheal sap may contain large concentrations, possibly because of the inability of the mottled leaves to utilize it.

3. Although no lithium is at present known to occur in citrus leaves, the success in the artificial production of mottle-leaf by the use of this toxic substance adds to our understanding of the mottle-leaf phenomenon.

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ORIGIN AND DEVELOPMENT OF TISSUES IN ROOT OF SCHIZAEA RUPESTRIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 392

DORR RAYMOND BARTOO

(WITH TWELVE FIGURES)

Introduction

The rhizome of *Schizaea rupestris* is about 2 inches in length and is densely covered with reddish brown hairs. The five or six sterile leaves are linear, with attenuated bases and apices, and attain a length of 3 or 4 inches. The fertile leaves, borne on the same rhizome, are pinnately divided and somewhat longer stalked than the sterile ones. The sporangia are borne singly in two rows upon the abaxial side of the pinnae. Both the fertile and sterile leaves exhibit the characteristic vernation of ferns. The rhizome branches occasionally, apparently dichotomously, while leaves and adventitious roots arise from its meristematic region. The roots are quite generally branched and attain a length of about 2 inches (fig. 1).

FIG. 1.—Habit sketch of mature plant: *s*, sterile leaves; *f*, fertile leaves.

The material upon which this study was based came from Sydney, Australia. Collections were made about November 1, when the growth of the plants was most vigorous.

The aim of this investigation was to determine the origin and development of the tissues of the root. Since all the cells of the root have a common origin in the apical cell, the study resolved itself into tracing the sequence of cell division from the immediate derivatives of the apical cell to the cells of the mature tissues. The various

structures of the root not only stand out definitely from one another, but each tissue is composed of an unusually small number of cells, enabling one to trace their lineage to the mature structures with remarkable certainty.

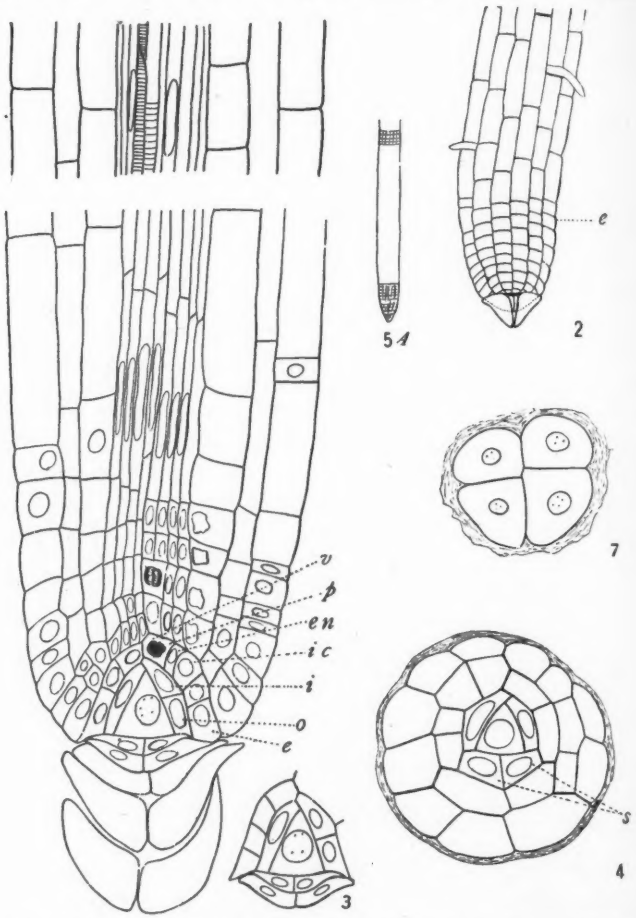
Apical cell and derivatives

The root has an average length of 5 cm. and a diameter of about 0.2 mm. All the tissues arise from segments cut from a pyramidal apical cell (fig. 3); that is, the apical cell is a triangular pyramid having its longest axis in the axis of the root (figs. 3, 5). It is about one and one-half times as deep as it is broad. The segments are cut off in serial order from its four cutting faces, the derivatives from segments of three of the faces becoming tissues of the root proper, while segments cut from the fourth face form the root cap.

The lineage of the successive segments may now be traced into the distinct tissues of the mature root. As viewed in longitudinal section, each of the immediate segments divides first by a periclinal wall into an outer and an inner cell (fig. 5 *o*, *i*). The outer cell, by one periclinal division, gives rise to an epidermal initial (*e*), and an initial of the outer layer of the cortex (*c*). The inner cell (*i*) by two successive periclinal divisions gives rise to four cells, the outer three of which initiate the inner layer of the cortex (*ic*), the endodermis (*en*), and the pericycle (*p*) respectively. It is from the innermost of the last cells (*v*) that the desmogen strand arises (fig. 5), but the subsequent behavior of this cell depends upon its radial position. Consequently, in addition to a consideration of the sequence of cell division as it appears in longitudinal section, the cell divisions occurring in the meantime as seen in transverse section must be borne in mind.

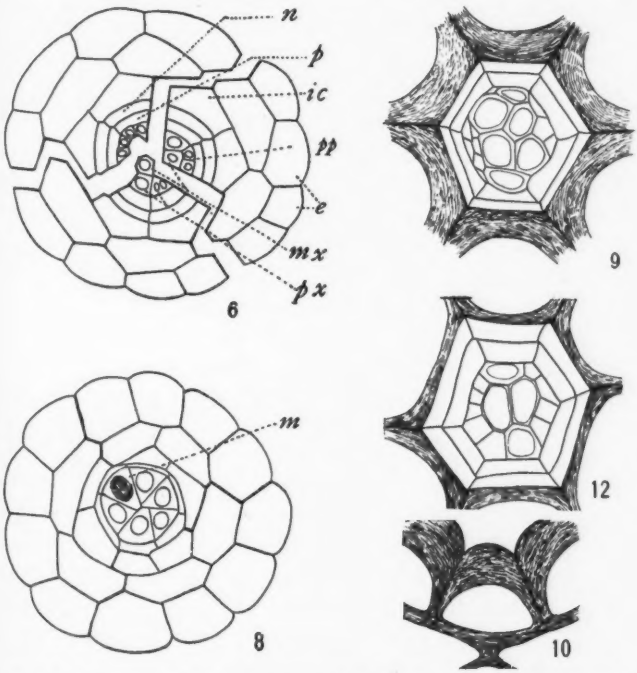
As just stated, each of the three immediate segments cut from the apical cell divides by a periclinal wall into an outer and an inner cell (fig. 5 *o*, *i*). Each of the three inner cells thus formed divides by a radial wall which extends from near the middle of the outer side inward, meeting the side of the segment near its inner angle. Thus is formed a sextet of cells of which the alternate three meet in the center while the remaining three do not extend to the center. This

dissimilarity of the inner sextet of cells is maintained in those subsequent derivatives which occupy similar positions at the center



FIGS. 2–5, 7.—Fig. 2, Habit sketch of root tip: *r*, root cap; *e*, epidermal cells; fig. 3, longitudinal section of apical cell; fig. 4, transverse section of apical cell; fig. 5, longitudinal section of root showing origin of tissues: *e*, epidermis; *c*, outer layer of cortex; *ic*, inner layer of cortex; *en*, endodermis; *p*, pericycle; *v*, desmogen strand initial; fig. 5*a*, longitudinal sketch showing relative position of two parts of fig. 5; fig. 7, transverse section of root cap near apical cell.

(fig. 8). Simultaneous with the division of the inner cell occurs the radial division of the outer cell. At this stage, then, we have a sextet of isodiametric cells inclosing a sextet of cells somewhat tetrahedral in form, as seen in transverse section. During the two successive



FIGS. 6, 8–10, 12.—Fig. 6, transverse section showing same tissue layers as fig. 5; fig. 8, transverse section of region of maturation; *m*, mitotic figure initiating pericycle and desmogen; fig. 9, transverse section through mature region: fig. 10, transverse section showing thickened wall of inner cortical cells; fig. 12, transverse section through mature region of root: *px*, protoxylem; *mx*, metaxylem; *pp*, phloem; *p*, pericycle; *n*, endodermis; *ic*, inner cortical layer.

periclinal divisions of each of the inner sextet, which immediately follows, each of the cells of the outer sextet also divides periclinally. These last periclinal divisions are followed by a radial division of each of the six cells of the outer layer, which completes the initial cells of each tissue layer (fig. 6). There are twelve cells in the epi-

dermis; six cells each in the two layers of the cortex, the endodermis, pericycle, and desmogen. No attempt was made to include, in this sequence of cell divisions, those occurring anticlinally. In general the anticlinal divisions of the epidermis, cortex, endodermis, and pericycle were several (six to ten); while probably never more than one occurs in the desmogen strand.

The subsequent behavior of each of the six desmogen strand initials depends upon its radial position. Two of the alternate three, which occupy positions at the center, divide periclinally and the two daughter cells which maintain the central position are metaxylem initials (fig. 6 *mx*), while the remaining two are destined to become phloem (*pp*). Two of the alternate three which do not occupy the central position become protoxylem initials (*px*). Not infrequently either the metaxylem initials or the protoxylem initials divide again, giving four metaxylem or four protoxylem strands. No cases were noted, however, where both the protoxylem and metaxylem initials divided in the same bundle. Each of the two remaining cells of the desmogen sextet divides once radially, and these initiate the phloem parenchyma (fig. 6); thus a diarch bundle is formed having four adjacent xylem strands, the two outer ones being protoxylem (*px*), and the two inner ones metaxylem (*mx*). It is to be noted that from each of two of the three original segments cut from the apical cell (fig. 4), both xylem and phloem arise, while the third gives rise to phloem only.

ROOT CAP

The root cap is produced by successive generations of segments cut off from the fore face of the apical cell. By two successive divisions, perpendicular to each other and also to the fore cutting face, four cells result (fig. 7). These cells grow to a considerable size but do not divide again. The cells from as many as four initial segments were observed on vigorously growing roots (fig. 5). The older cells are roughly pear-shaped and inflated on the side away from the growing point. As they become separated from the food supply their contents become less and less dense, and partial disintegration of their walls often takes place before they are sloughed off.

As is shown in both transverse and longitudinal sections, the tissues are extremely diagrammatic. The constancy of the number

of cells per layer as seen in transverse section is a striking feature; twelve for the epidermis and six each for the cortex, endodermis, and pericycle layers. While the number of phloem parenchyma strands varies from eight to twelve, the xylem almost always consists of four or six elements (figs. 9, 12). All the tissues of the root are differentiated very early. The initial cells for the epidermis, cortex, endodermis, and pericycle are only two cells removed from the apical cell, while xylem and phloem strands can be recognized soon after.

Epidermis

For a distance of 0.2 mm. behind the root cap there occurs rapid cell division anticlinally (fig. 5). While most of the cells of the epidermis are isodiametric, some of them remain flattened, with their longitudinal axes much shorter than those of their neighbors (fig. 5). These smaller cells, which are destined to produce root hairs, cease to divide and can be recognized when only six cells removed from the apical cell. They do not elongate in the direction of the axis of the root, as do the other epidermal cells, but even before their sister cells have ceased to elongate their volume is increased by their extension into root hairs. Of the seventy-five root hairs observed, there were no exceptions to the fact that the epidermal cells producing root hairs do not increase to any appreciable extent except by means of extending root hairs. From differences in staining reactions it seems probable that there is a difference in density, and perhaps also a chemical difference in these cells some time before root hairs are produced. Less than one-tenth of the epidermal cells produce root hairs, even though the latter occur upon roots throughout their entire lengths.

Cortex

The cells of the cortex keep pace in their divisions and elongation with those of the epidermis. The cell walls of the inner layer become greatly thickened upon their inner and radial faces. Prantl (5) refers to these thick-walled cells in *Schizaea pennula* as "die sechs dickwandigen inner Rindenzellen." Miss Eckerson found by microchemical tests that the corresponding thick walls in *S. pectinata* consist of cellulose with suberized and lignified lamellae (fig. 10). Thickenings on the cortical walls next to the epidermis are first evi-

dent about 0.6 mm. from the apical cell. The thickened area then spreads inward until it includes all the cells of the cortex and those of the endodermis. The thickenings to this point (0.8 mm.) are thin and uniform, but beyond this thickenings upon the inner and radial walls of the inner cortical cells continue until the lumen is reduced to at least two-thirds of its original size.

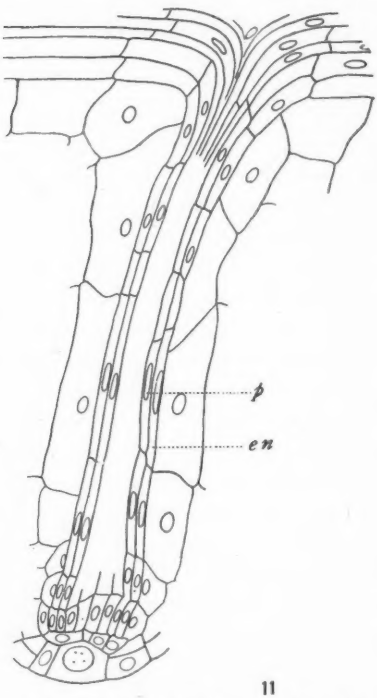


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FIG. 11.—Longitudinal section of root rising from rhizome: *en*, endodermis; *p*, pericycle.

ENDODERMIS

The endodermis and inner layer of the cortex arise from a common mother cell (fig. 5). They are of equal size at first but the cortical cells soon become noticeably larger. Casparian strips were not observed, but the continuity of these cells of the fourth tissue layer (*en*) with the endodermal cells of the rhizome was easily traceable (fig. 11). PRANTL figures the corresponding endodermal layer for *S. pennula*, while Miss ECKERSON finds that the corresponding layer in *S. pectinata* shows "casparian flecks."

PERICYCLE

The pericycle cells are sister cells to those of the desmogen strand. It was noticed that their radial walls remain in the same planes with those of the endodermis and the inner cortical layer (fig. 6). The primary walls of the desmogen strand likewise remain in the same planes. This arrangement of cells might well be expected, since each tissue layer is composed of the same number of cells and since all have a common ancestor (fig. 5 *i*).

Phloem

Adjacent to the inner side of four of the six pericycle cells arise the phloem parenchyma initials. From serial transverse sections it was impossible to distinguish sieve tube elements in the phloem parenchyma. At a distance of 0.5 mm. from the root cap the cells are uniform in size, but at a distance of 1 mm. larger ones were recognizable. It is quite probable that these larger cells are of the metaphloem, while the protophloem sieve tubes remain indistinguishable from phloem parencyhma. No sieve areas were observed in the phloem tissue.

Xylem

As previously stated, the arrangement of the bundle is diarch, and the xylem usually consists of two protoxylem and two metaxylem cells. Each protoxylem cell lies adjacent to the metaxylem cells. At first these sister cells are similar in size and structure, but at a distance of 1 mm. from the root cap those of the metaxylem have considerably larger lumen and more rarefied contents. Wall thickenings were observed in protoxylem 0.7 mm. and in metaxylem 0.9 mm. from the apical cell (fig. 5).

In the region of elongation the desmogen strands are greatly elongated, while the cells of the cortex remain more nearly isodiametric in form. As pointed out by Chang (3), mitotic activity ceases in the transverse plane of desmogen cells while they are still in the isodiametric form. They lengthen while their neighbors divide transversely, reaching a length of ten to twenty times their diameters (fig. 5). This is in agreement with the unpublished work of Land, in which he states that the desmogen initials are stretched out because of the transverse divisions of the cortical cells.

Discussion

The root structure of *Schizaea rupestris* is probably the simplest and most diagrammatic of all observed roots. A transverse section of a mature root shows only three layers of cells outside the endodermis; the epidermis consisting of twelve cells and two layers of cortical cells each consisting of six cells. The single-layered endodermis and pericycle consist of six cells each. The xylem is usually of four cells (sometimes six), while the phloem varies from eight to

twelve cells. BOODLE (1) describes *S. digitale* as having twelve cells each in its epidermis and outer cortex, while the inner layer of the cortex (sclerotic cells) varies in number from the usual six to eight or nine. BRITTON and TAYLOR (2) find that the number of cells per layer corresponds in general to that of *S. digitale* and to *S. pennula* as described by PRANTL.

BOODLE finds that the radial walls of the pericycle, endodermis, and "sclerotic layer" of the cortex of *S. digitale* lie in the same radii, while the walls of the next cortical layer, which may consist of twelve cells, do not coincide with these radii. He states that the pericycle therefore appears to be cortical.

On the grounds that the endodermis and pericycle usually rise in common with the cortex, RUSSOW (6) proposed to exclude these tissues from the vascular system. STRASBURGER applied the term "phloeterma" to the inner layer of the cortex (endodermis), and considered all the tissue external to it as cortical. The recent work of CHANG on the rhizome of *Pteris aquilina* shows that the endodermis and pericycle have a common origin, and that both layers are stelar. TANSLEY and CHICK (8) state that they can hardly imagine that the sheath layers (endodermis and pericycle) are not phylogenetically identical in monostelic ferns. CONRAD (4), working on *Dicksonia punctilobula*, found that the endodermis of the root is cortical in origin, while that of the rhizome is stelar. He gives a tabulated list of thirty-four genera of ferns in which the endodermis of the roots is said to be cortical in origin.[1]

PRANTL suggests that the endodermis in *Schizaea*, "just as in *Hymenophyllum*," is a part of the vascular system, for the reason that they have in many cases "without doubt" the same origin as the pericycle. He found that the endodermis and pericycle have a common origin in the smallest leaf veins of *S. pennula*.

In the present study the pericycle and endodermis are clearly of different immediate origin. The endodermis and inner layer of the cortex arise from a common initial which is sister to the cell giving rise to the pericycle and vascular elements. This is in accordance with HANSTEIN'S histogenetic idea of plerome, periblem, and derma-

[1] Mostly from works of VAN TIEGHEM, DOULIOT, NAGELI, and LEITGEB.

togen. The endodermis constitutes the inner layer of the cortex and the pericycle is the outer layer of the stele.

If the distinction between stele and cortex is a morphological one, the two regions must be marked off from one another in the earliest periclinal cell divisions of the original apical cell segments. Research upon the apical cell meristem of ferns by CHANG, CONRAD, and TANSLEY (7) shows that these earliest divisions of the apical cell derivatives are inconstant; therefore this means of differentiating subsequent adult tissues is unreliable.

Although the findings of the present investigation support HANSTEIN's histogenetic idea of periblem and dermatogen, the data here presented indicate that histological distinctions are not trustworthy as a means of differentiating subsequent adult tissues of the plant.

Summary

1. All the tissues of the root have their origin in a pyramidal apical cell.

2. The root structure is probably the simplest of all observed roots.

3. Tissues of the root are differentiated extremely early; the initials of the epidermis, cortex, endodermis, and pericycle are only one cell removed from the apical cell.

4. The inner cortical layer and endodermis have a common origin; both are cortical.

5. The pericycle and desmogen strand have a common origin; both are stelar.

6. The arrangement of the bundle is diarch, consisting of four or six xylem cells and from eight to twelve phloem cells.

7. Thickening of the inner and radial walls of the "sclerotic layer" is a striking feature.

8. Epidermal cell of root hair destiny can be recognized very close to the apical region, and root hairs persist throughout the life of the root.

9. Histological distinction is unreliable as a guide to adult tissues.

The writer acknowledges with pleasure his indebtedness to Professor W. J. G. LAND, under whose direction this investigation has been conducted. He also appreciates the helpful suggestions given by Professor C. J. CHAMBERLAIN. Thanks are due to Mr. PATRICK BROUGH of the University of Sydney, who made several trips to collect and fix the material used in this investigation.

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PROGENY RESULTING FROM SELF-POLLINATION OF STAMINATE PLANT OF MORUS ALBA SHOWING SEX REVERSAL[1]

JOHN H. SCHAFFNER

In 1925, the writer (3) reported some observations on the nature of the sexual expression of ten white mulberry trees which were grown from seed obtained from a reversed branch of a staminate individual. These seeds were developed through open pollination. Of the ten trees, seven were carpellate and three staminate. Later, two of the carpellate plants showed some staminate flowers and one of the staminate plants also developed a slight amount of femaleness. The tree from which the seed was obtained showed a very interesting condition, in that one large branch developed the partial sex reversal while the other branches were typically in the secondary male state. The branch continued to produce staminate, carpellate, and mixed catkins for a number of years, until the tree was destroyed.

In the spring of 1924, a staminate tree near the botany-zoology building on the Ohio State University campus showed sex reversal in several small branches. Two of these branches were promptly covered with cloth, with some staminate branches included to insure self-pollination. A small quantity of self-pollinated seed was procured, from which fifty-five seedlings were developed in the botanical greenhouse. These little trees were transplanted out of doors in the spring of 1925, and thirty-two of them survived until the spring of 1928. In 1927, two of these trees bloomed and both were pure staminate in expression. Both of these plants bloomed again in 1928, along with four others, making a total of six in bloom. One of the plants which bloomed in 1927 was again pure staminate, while the other was prevailingly staminate but with several carpellate or partly carpellate catkins. The four new trees to come into bloom were as follows: two were pure carpellate in sexual expression; the other two were carpellate, each with a few staminate flowers.

[1] Papers from the Department of Botany, the Ohio State University, no. 226.

These six trees, therefore, derived from a selfed staminate plant, showed all the possible general sex conditions: pure female, pure male, female with reversed branches, and male with reversed branches. The potentialities which were transmitted showed that the original male condition of the parent tree possessed all the essentials for both maleness and femaleness. The development of maleness in the original staminate parent, therefore, was not due to any differential factor or chromosome condition of the cells; for maleness changed to femaleness in several vegetative branches through the ordinary somatic karyokinesis. And since the same branches also continued to produce staminate catkins or to revert back to the staminate condition, the change was not due to the loss of a chromosome or to the crossing over of a gene from one system to the other. The reduction division which segregated the chromosomes with the hereditary factors took place after sex reversal had been accomplished; and as is well known, the sex of the gametophyte was not disturbed or changed by the shifting of the heredity, but was continued in the same condition as the branch from which the gametophytes had their origin.

It is possible that no reduction took place and that the eggs developed parthenogenetically, of course; but if this were the case, it is evident that the several kinds of sex determinations were no more dependent on chromosomes or factor combinations or balances than if the reproductive processes are assumed to have developed normally.

It is now desirable that experiments be carried on with selfed carpellate plants; but however these may turn out, we know that the carpellate plants also frequently undergo sex reversal in some of their branches, giving rise to functional staminate flowers (2). Thus just as in the case of the staminate plants, the original sex condition of the carpellate individuals was not established because of the absence of a potentiality to produce a male state, nor any of the hereditary factors necessary for the development of all the normal secondary and primary male states and characters. The original female condition in such cases was plainly established in the presence of such factors. Both the original zygotes from which the staminate and carpellate plants develop have complete potentiality for

the production of maleness and femaleness. The difference in sex determination and sex expression is therefore not due to any differential in heredity, but is brought about by some difference in functional level of the two zygotes involved. This difference in functional or physiological level is brought about through many different causes, among which are to be reckoned both the external environment and the internal food condition, together with the metabolic gradient of the egg at the time of fertilization. The sex reversals which occur in the developed and differentiated organism are brought about by the same conditions in the presence of the potentialities for both the male and female states. These changes are brought about in spite of a certain sex differentiation as well as somatic differentiation, which has taken place in the entire plant, and which is often evident in the external morphology as well as in the deeper physiological processes, involving catalase and other activities.

In *Mercurialis annua*, YAMPOLSKY (9) found from limited experiments that maleness tended to perpetuate maleness and femaleness tended to perpetuate femaleness; in that his self-pollinated, reversed, staminate individuals produced male offspring and his reversed, carpellate individuals produced female offspring. *Mercurialis* evidently has a hereditary constitution of such a nature that the eggs and sperms coming from an original male soma continue in a high metabolic state which prevents the resulting zygote from falling over to the female condition, at least under the ordinary environments employed; while the eggs and sperms coming from an original female soma do not rise to the metabolic level in which the sexual state could fall to maleness.

HIRATA (1) carried on a considerable number of experiments by selfing reversed hemp plants, and also by crossing reversed plants with each other. From his experiments it is evident that a considerable percentage of the seeds did not sprout, and some, as would be expected, failed to reach maturity. A summary of his main results, including the F_1 progeny, is shown in table I.

Although less than 60 per cent of the seed planted came to maturity, the results seem to indicate that females tend to produce females while males tend to produce both males and females. The

experiments on the latter proposition are too few to give definite conclusions. The experiments do show, however, that both males and females come from either condition. The great predominance of female intersexes in experiment 3 was no doubt due to the time of planting, since the dates given run from December to January. The writer has shown that the percentage of "pure individuals" to "intersexes" is dependent on the environment in which the plants develop. One can keep the "intersexes" at zero or raise them to 90 per cent or more at will. Genetically it is of no importance whether the individual for the time being is unisexual or bisexual

TABLE I

	Seeds planted	Seeds sprouted	Females	Female intersexes	Males	Male intersexes
1. Female intersex selfed	294	143	78	45	3	0
2. Mutual crossing of female intersexes	48	24	12	4	1	0
3. Mutual crossing of female intersexes	345	242	48	186	0	0
4. Female×female intersex	50	41	27	8	1	0
5. Female×female intersex	55	48	37	8	0	0
6. Female×male intersex	89	59	28	0	6	22
7. Female×male intersex	16	13	4	0	3	3
8. Female intersex×male intersex	38	25	13	0	9	0
9. Male intersex×male intersex	18	10	6	0	2	0

in expression, except that the bisexual condition gives an opportunity for selfing. Individuals that happen to be developed as pure male or pure female can be brought to the bisexual condition through proper rejuvenation treatment (7).

HIRATA'S experiments plainly show that sex determined in the seed is not due to any homozygous-heterozygous shifting of chromosomes or sex heredity; for just as staminate plants can change to carpellate and carpellate to staminate after their sex has been determined, so a hereditary constitution which is determined as male can produce both male and female individuals through the zygote, and a hereditary constitution which is determined as female can in the same way produce both male and female individuals. The breeding experiments on hemp as well as the fact of the abundance of reciprocal sex reversal, which is so easily brought about under proper ecological conditions, show conclusively that no matter what the

chromosome constitution of hemp may be, the facts do not lend themselves to any plausible explanation on the basis of the hypothetical "XY type" of inheritance.

In hemp, as in the mulberry, it is no more a question of determining whether one or the other sex is homozygous or heterozygous (we know that they are both heterozygous if the word means anything), but it is a problem of great interest to discover the functional conditions of the gametes produced on the two sexes and the sex ratios of the resulting progeny.

As intimated, it is evident that in all cases of sex reversal in monosporangiate species, like hemp, Japanese hop, Jack-in-the-pulpit, tall meadow-rue, etc., the original development of the given sexual state cannot be ascribed directly to any differential heredities they may contain, nor to any chromosome differences, since at a later stage of the ontogeny, the very same hereditary constitution and the very same complement of chromosomes give rise to the opposite sex expression. In terms of the Mendelian hypothesis of sex, the chromosomes or the hereditary factors would thus be determining exactly the opposite from what they had done before. It must be evident that if the physiological condition of the cell or tissue can re-determine the sexual state and the sexual characters after differentiation in the original sexual state has been accomplished, there is no basis for a blind faith in the assumption that in the zygote the sex was not determined in exactly the same way, but by the special set of allosomes or sex genes present. If these were the causal agents in the zygote, why are the so-called "sex chromosomes" found to be entirely passive at the reversal stage? It must be recognized that the Mendelian sex hypothesis was an assumption in the first place, which needed proof from all unisexual species for its serious consideration. The mere coincidence, in certain cases, of definite allosome sets with definite sexual states does not prove that the two have the relation to each other of cause and effect, even if such correspondence were universal, which is very far from the facts of the case. In the higher plants the correspondence of unisexuality of the individual with a special allosome condition is comparatively very rare, and for the gametophyte there is no correspondence at all.

Studies on the resulting sexual states from selfed reversed individuals should be extensively made, for the results will lay a firm foundation of fact on which to establish a fundamental theory of sex determination. The experiments carried on so far indicate that quite different results are to be anticipated from different hereditary constitutions reacting in normal environments as well as in abnormal ones. Whatever the actual results, the interpretations must be in harmony with the conditions found in general for the species. This has not been done heretofore. It is also evident that if changing physiological states can bring about complete sex reversal in the soma, similar reversals can also be accomplished in the zygote. The only problem is to find efficient ecological factors to induce the proper functional states.

Summary

1. A reversed staminate tree of *Morus alba* was self-pollinated and produced good seed from which thirty-two young trees were grown. Of these the first six to bloom had sex expression as follows: one tree was pure staminate for two years; one was pure staminate the first year and of mixed sex the second year; two were pure carpellate; and two were carpellate with a slight development of staminate catkins.

2. Determination of the sex of the original male parent, therefore, was not due to any sex-determining differential heredity present, since it proved to have the potentialities for both sexes, as shown by the reversal in its soma; and the sex of the offspring was also determined in the zygote as male and female, through a physiological gradient which caused a male or a female state to be differentiated in the same manner as and through the same cause as that which induced these states to develop through sex reversal in the advanced ontogeny of the individual. Sex-determining factors and sex-determining chromosomes can have no correspondence with the phenomena brought out by the experiment.

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IRREVERSIBLE INJURY AND CO_2 PRODUCTION FROM CELLS OF NITELLA FLEXILIS

P. A. DAVIES

Introduction

It is a well established fact that CO_2 is produced abnormally in stimulated and injured tissue. The mechanism of CO_2 production by "dead" tissue is in doubt. To say that a cell is dead because it fails to meet certain criteria may be erroneous, for the cell may be injured to such an extent that it is unable to respond to certain tests, and yet retain certain vital properties. HAAS (**3, 4**), using *Laminaria* tissue, found an increased rate (above the normal) of CO_2 production from dead cells, death being determined by a conductivity method (described by OSTERHOUT **8**). Irreversible injury and death are not synonymous terms, for irreversible injury may be considered to occur at that point on a curve of injury beyond which the cell fails to recover beyond a certain point; while death signifies complete inability to recover to any degree.

This study is concerned primarily with the production of CO_2 from irreversibly injured cells of *Nitella flexilis*.

Material and methods

The cells of *Nitella flexilis* were rigid and separated freely from one another, so that a large number could be used with assurance of complete circulation around each cell. The number used for each experiment varied between 75 and 125 large cells, with an approximate average of 6.23 smaller rosette cells for each large cell.

The apparatus used was that evolved by OSTERHOUT (**9**). Before being placed in the apparatus, all material was first brought to the desired temperature (30° C.). In every experiment a normal rate of CO_2 production was established before the reagent (ethyl alcohol) was added. In order to consider the production of CO_2 by cells irreversibly injured, the irreversibility of the injury must be assured. In establishing a criterion of such injury the following methods were employed: (1) loss of rigidity of the cellular struc-

ture;[1] (2) cessation of cytoplasmic streaming; (3) inability of the cell to accumulate cresyl blue; and (4) non-recovery under optimum conditions. The writer found that in every case where the rigidity of the cell had disappeared cytoplasmic streaming had stopped,[2] the cell failed to accumulate cresyl blue, and no recovery took place after seven days under optimum conditions. The loss of rigidity is therefore taken as an index of irreversible injury.

Results

In working with uniform material under constant conditions, variations were found in the time of exposure to a definite concentration before the appearance of irreversible injury (loss of rigidity). These variations must be due to differences among the cells.[3] Table I shows the time required for cells to lose their rigidity in different concentrations of alcohol. It will be observed that the time required for cells from the same lot of material to lose their rigidity varied greatly; for instance, in the 10 per cent solution a variation latitude of 20 minutes and 19 seconds is shown. As the concentration increases, the latitude of variation becomes less. Due to the variation of material, a variation in CO_2 production was obtained; so that each average shown (table II) represents the average of three to thirteen experiments.

When alcohol was added to the material in the apparatus (after a normal rate of CO_2 production was established), an initial rise in the rate of CO_2 production was induced. This rise is not specific for the action of alcohol on cells of *Nitella flexilis*, for such a rise has been observed by IRVING (5) when young shoots of *Hordeum vulgare* were subjected to low concentrations of chloroform; HAAS (3, 4) when

[1] The loss of rigidity of the cell was determined by means of a glass rod. One end of a glass rod 5 mm. in diameter was drawn out into a smaller diameter (1 mm.) and this was bent into a hook. If a cell bent across this hook so that when removed from the solution the free ends touched and failed to separate, the rigidity of the cell was considered lost.

[2] KÜHNE (6) and NOTHMANN-ZUCKERKANDL (7) found that cytoplasmic streaming stopped without recovery when the cells of *Nitella* were subjected to high concentrations of narcotics.

[3] The material was kept in the laboratory in an aquarium in which the water was changed daily, the temperature of which was approximately 18° C. A new lot of the material was secured every 10–12 days.

alcohol, acetone, ethyl chloride, and formaldehyde were added to *Laminaria* tissue; GUSTAFSON (2) with formaldehyde, ether, and acetone on *Aspergillus niger;* BROOKS (1) with ether solution on *Bacillus subtilis;* and RAY (10) when *Ulva* tissue was subjected to low concentrations of chloroform.

The height of the initial rise in CO_2 production depends on the concentration of the alcohol. A 20 per cent solution caused the highest initial rise, followed by the 10, 30, 40, 60, and 95 per cent solutions respectively. In the 10 per cent solution, the alcohol did not penetrate the cell structures rapidly enough (determined by the

TABLE I

TIME REQUIRED FOR CELLS OF NITELLA FLEXILIS TO LOSE RIGIDITY IN DIFFERENT CONCENTRATIONS OF ETHYL ALCOHOL; TEMPERATURE 30° C.

PERCENTAGE ETHYL ALCOHOL (BY VOLUME)	TIME REQUIRED FOR FIRST CELL TO LOSE RIGIDITY		TIME REQUIRED FOR LAST CELL TO LOSE RIGIDITY		AVERAGE TIME FOR LOSS OF RIGIDITY IN ALL CELLS TESTED	
	Minutes	Seconds	Minutes	Seconds	Minutes	Seconds
10	25	4	45	23	33	58
20	2	10	3	38	3	19
30	1	7	1	35	1	18
40	.....	30	.....	49	.....	39
60	.....	15	.....	22	.....	19
95	.....	4.5	.....	7	.....	5.9

time required for loss of rigidity) to cause a rapid release of CO_2 induced by the 20 per cent solution; in the 30, 40, 60, and 95 per cent solutions the alcohol caused a rapid change of the cell structure to such an extent that CO_2 was released slowly from the irreversibly injured cells, and was taken up by the solution as it escaped, according to the coefficient of CO_2 absorption by the solution.

The OSTERHOUT apparatus is so constructed that only when the CO_2 equilibrium of the system is established is the rate produced by the material accurately measured. With the sudden increase (initial rise) in the rate of CO_2 production, the CO_2 equilibrium is disturbed to such an extent that the production of CO_2 after the initial rise cannot be measured accurately until reestablishment of the equilibrium. The CO_2 measured between the disturbance and reestablishment of the equilibrium is the CO_2 produced by the initial rise plus the CO_2 normally produced after the initial rise.

If irreversible injury takes place before the reestablishment of the CO_2 equilibrium, the measured CO_2 at that time will show a higher rate than is produced by the material. The high rate would cause a delay in the drop of CO_2 production below the normal. In all concentrations, except the 10 per cent solution, irreversible injury occurred before the reestablishment of the CO_2 equilibrium.

Table II shows a comparison of the average time for loss of rigidity (irreversible injury) and the average time for CO_2 production to drop below the normal rate. It will be observed that the greatest delay (time between irreversible injury and drop of CO_2

TABLE II

COMPARISON OF AVERAGE TIME FOR LOSS OF RIGIDITY AND AVERAGE TIME FOR CO_2 PRODUCTION TO DROP BELOW NORMAL RATE AFTER ALCOHOL WAS ADDED

PERCENTAGE ETHYL ALCOHOL (BY VOLUME)	AVERAGE TIME FOR LOSS OF RIGIDITY IN CELLS TESTED		AVERAGE TIME FOR CO_2 PRODUCTION TO DROP BELOW NORMAL AFTER ALCOHOL ADDED		DIFFERENCE BETWEEN TIME FOR LOSS OF RIGIDITY AND TIME FOR CO_2 PRODUCTION TO DROP BELOW NORMAL	
	Minutes	Seconds	Minutes	Seconds	Minutes	Seconds
10	33	58	32	16	1	42
20	3	19	8	30	5	11
30	1	18	3	12	1	54
40	.....	39	2	13	1	34
60	.....	19	1	22	1	3
95	.....	5.9	.....	53	.....	47.1

production below the normal rate) occurred in the 20 per cent solution; also, that as the concentration increased the delay became less. As already explained, the highest initial rise occurred in the 20 per cent solution; and as the concentration increased, the height of the initial rise decreased. It would appear, therefore, that the delay in the drop of CO_2 production below the normal rate must be due to the CO_2 produced by the initial rise. In the 10 per cent solution, the period between the addition of alcohol and irreversible injury was of such a magnitude that the CO_2 equilibrium was established before irreversible injury occurred, so no delay was noticeable.

The data apparently indicate that the rate of CO_2 production drops below the normal at the time of, or very shortly after, irreversible injury occurs. The findings are not in agreement with those of HAAS (3, 4); for he found the rate of CO_2 production by *Laminaria*

tissue to be higher than the normal for an extended period after the cells were dead.

Summary

1. The data would seem to indicate that the rate of CO_2 production from cells of *Nitella flexilis* drops below the normal at the time of, or very shortly after, irreversible injury occurs.

2. The findings are not in agreement with those of HAAS, who found an increased rate (above the normal) of CO_2 production by *Laminaria* tissue for an extended period after the cells were dead.

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BRIEFER ARTICLES

ARTIFICIAL CULTURE OF GANODERMA LUCIDUS LEYSS FROM SPORE TO SPORE

(WITH ONE FIGURE)

Ganoderma lucidus is a saprophyte as well as a wound parasite. COLEMAN[1] has recently made a very thorough and interesting study of the nature of the spore wall in this species. He states that after repeated attempts he could not obtain a single germination of its spores.

For purposes of spore culture, a fresh sporophore was collected in February, 1927, from the suburb of Calcutta. It was thoroughly washed in sterilized distilled water. A petri dish containing sterilized malt extract agar medium[2] was taken, and on the inner surface of the upper lid a small piece of the sporophore was attached by means of melted agar in such a way that the porous surface remained pointing downward above the medium, when the upper lid was put in position. The petri dish in this condition was kept within a bell jar lined by water-soaked blotting paper to maintain the proper humidity of the inclosed air. The next day the petri dish was removed from the bell jar and examined under the low power of the microscope. It was found that a number of spores had dropped on the medium, and that some had germinated into mycelium.

Various media, such as wood decoction agar, prune agar, etc., had been tried for germination of spores of this fungus, before it was finally found that spores could be germinated in the malt extract agar medium, with a pH of 6.9. At once mycelial transfers were made aseptically by means of a platinum loop to a number of sterile tubes containing the same sterilized medium. These were kept within a glass chamber from February to August. During this period the room temperature varied from 77 to 95° F., and the relative humidity of the glass chamber varied from 62 to 74 per cent. At first growth was rather slow. In the course of about a month and a half the whole of the slants became covered with white fluffy growth, which gradually thickened, and there was exudation of a number

[1] COLEMAN, L. C., Structure of spore wall in *Ganoderma*. BOT. GAZ. **83**: 48–60. 1927.

[2] Agar 2 per cent, malt extract 3 per cent, water 100 cc.; agar for this purpose was washed overnight in running water, then washed in distilled water and boiled.

of small glistening drops from the white hyphae and a number of crystals of calcium oxalate were found on the hyphae. No discoloring of the malt extract agar medium was noticed, nor any formation of conidia. The hyphae were closely septate and much branched, and clamp connections were quite common. Growth within the tubes declined at the end of about two months from the date of inoculation, and continuous subcultures to fresh tubes with the same medium were carried on almost every month from March until the present time. In this way the growth has been kept up. Some of these subcultures showed the formation of loose pores without any pilear form on the white mycelial surface, within which typical mature brown spores of *Ganoderma lucidus* were found after an interval of about a week from the date of inoculation.

FIG. 1

In April, 1927, mycelial transfer was made from one of the tubes to a sterilized block of dead but compact wood of *Mangifera indica* within a sterile Roux tube with a little sterile water at the constricted end. This tube was kept at ordinary room temperature within the same glass chamber. The mycelial growth on the block of wood was poor, the growth being confined to the upper part of the block. The wood showed no external sign of decay. During July the white mycelial mat on the top surface of the block gradually acquired a pinkish tinge, while the outer brim showed a number of pore formations (irregular hymenial patch), the white pores being pointed upward. By the end of August, that is, after an interval of about five months, a tiny fructification in the form of a pinkish-white hemispherical hump was formed on the top of the wood block (fig. 1). It showed three concentric rings in the form of a zonation on the upper surface, which was gradually acquiring a deeper tint. The fructification resembles in form the cushion-like expansion figured and described by WHITE.[3]

A fragment taken aseptically from the top surface of the wood block by means of a platinum loop, and examined under the microscope on

[3] WHITE, J. H., Biology of *Fomes applanatus* (Prs.) Wallr. Trans. Royal Canadian Inst. Toronto. 1919.

August 26, showed the formation of a number of typical spores of *Ganoderma lucidus*. The color of the spore wall was not quite brown, however, not being fully mature, and showed a lighter (yellowish) shade. I could get the spores at different stages as figured by COLEMAN in the article already referred to. Soon after, the deposit of spores on the sides of the Roux tube could be distinctly seen in the form of a cloudy mass.

Mycelial bits from a pure culture were also transferred to sterilized wood blocks of *Spondias mangifera* and *Tamarindus indica* in August, 1927. One of them, that on *Spondias mangifera*, has given rise to a number of typical spores, although no regular sporophore has been formed as yet. Both of these wood blocks show poor mycelial growth on their upper part. They are exuding copiously glistening drops of liquid.

It is my experience with the artificial cultures of a number of fleshy fungi that the fruiting bodies hardly assume the normal form. This might be due to the artificial conditions of restricted air supply, light, and moisture, under which they grow.

Incidentally, while studying the cytology of the basidia of some sporophores of this species, I was able to demonstrate by GOLGI's bichromate and silver-nitrate method a fine blackened net within the basidium, which recalls the nature of the GOLGI apparatus in animal cells.—S. R. BOSE, *Carmichael Medical College, Calcutta, India.*

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CURRENT LITERATURE

BOOK REVIEWS

Silviculture

In a recent volume TOUMEY[1] has shown the close harmony existing between silviculture and plant ecology. Ecologists are accustomed to say that all forestry is but applied ecology, but TOUMEY claims that "the foundation of silviculture as we conceive it today, is not an outgrowth of plant ecology, but rather plant ecology is an outgrowth of it," and it is doubtless true that silviculture was well established before ecology was recognized as a separate branch of biological science. It is also true that such work as that of EBERMAYER, begun in 1871, in forestry experiment stations, was good plant ecology of the truly scientific and experimental type. To these stations is credited the beginnings of the era of modern scientific silviculture.

TOUMEY credits plant ecology with many contributions to silviculture, the most important of which is the development of the concept that the plant community is a fundamental unit of vegetation. On the other hand, he says that plant ecologists have seized upon the methods of the silviculturist in the study and measurement of habitat or site factors. This seems an ideal cooperation, and this unity of aim and similarity of purpose in ecologist and silviculturist permeates the entire volume; in fact, ecologists would do well to study the book carefully and might profit by adopting it as one of their textbooks.

The author proposes to divide the discussion of his subject into three parts: site factors, forest vegetation, and methods of investigating site factors and forest vegetation and relating the one to the other. Only the first and second parts are included in the present volume; the discussion of the third will appear later.

The scope of part I may be gathered from such chapter headings as definitions and generalities; climatic factors; physiographic factors, and biotic factors; and reaction of forest vegetation on the site factors. The discussion of part II is under such heads as forest vegetational units and their classification; life history of forest communities; the stand; the tree.

While all topics are well discussed, particular mention might be made of the careful consideration of light and the definition and analysis of "tolerance." Excellent diagrams help to illustrate and to separate the combined effect of light and soil moisture on reproduction and undergrowth. Among the physiographic factors, forest humus and soil moisture receive very careful attention. In connection with the latter, TOUMEY'S own contribution on the characteristic forms of initial root habit and their relation to available soil moisture is very valuable

[1] TOUMEY, J. W., Foundations of silviculture upon an ecological basis. Vol. I. 8vo. pp. 438. *figs.* 11. New York: John Wiley & Sons. 1928.

and noteworthy. Biotic factors are more briefly discussed, mycorhizas in particular being dismissed with rather scant attention.

In the classification of forest communities, the familiar ecological systems of SCHIMPER and WARMING are sketched; and for North America those of SARGENT, CLEMENTS, SHREVE, and ZON are outlined in more detail. In the matter of forest types, the discussion becomes more strictly that of the forester, although much stress is placed on CAJANDER'S system of basing the classification on indicator plants found in the undergrowth of the forest. While this system is not without its difficulties, it seems about the best available to evaluate the potentialities of the site. In the life history of forest communities, the principle of succession is recognized and applied to problems of establishment and reproduction.

Throughout the entire volume the basic fundamental principles are sought and the discussion is clear and logical. The organization is excellent and the bibliography shows a thorough familiarity with ecological as well as with forestry literature. Its viewpoint and scope make it useful and instructive to the ecologist, and indispensable to the forester.

A second book on silviculture by HAWLEY[2] is from the same laboratory and by the same publishers, but presents a very different viewpoint. It deals with the practical application of the principles discussed by TOUMEY. Its aim is to direct the forester how to produce and manage a forest in such a manner as shall best fulfill the objects of the owner. It seems to discuss its problems with clearness and vigor, and to bring to the discussion the best technical knowledge available.

The contents of the volume may be judged by such chapter headings as: reproduction methods; clear cutting methods; seed tree method; shelter wood method; selection method; coppice method; intermediate cuttings; slash disposal; forest protection; fire control; protection against insects; and protection against tree diseases. Its organization is such that the topics follow one another in orderly succession, and it is well illustrated with diagrams and charts. An extensive bibliography is cited, the citations being arranged in lists at the end of each chapter, while an extensive index seems to make all topics readily accessible.

Both books are well printed and bound, and will form valuable additions to ecological and forestry classics.—G. D. FULLER.

Colloid chemistry

A remarkable collection of papers has been brought together in the second volume on colloid chemistry, edited by ALEXANDER.[3] This volume contains the

[2] HAWLEY, R. C., The practice of silviculture with particular reference to its application in the United States of America. 8vo. 2d.ed. pp. 335. *figs. 69*. New York: John Wiley & Sons. 1929. $4.

[3] ALEXANDER, JEROME, Colloid chemistry. Vol. II. 8vo. pp. 1029. Chemical Catalog Co., New York. 1928.

papers in the field of biology and medicine; the first volume was issued some time ago, and covered the general theoretical field. The third volume will consider industrial applications of colloidal chemistry and physics.

The book contains fifty-seven monographs on many different phases of colloidal behavior. Many of the authors have won international fame for their work in special phases of colloidal action, and it is an extremely valuable addition to the literature of colloids, mainly because of the character of the authorship. It is not possible in a brief review to give an adequate idea of the value of the various monographs. However, the names of some of the authors and titles of papers are mentioned to show the unusual character of the book. STÉPHANE LEDUC contributes a paper on solutions and life; Sir WILLIAM BRAGG discusses colloids and X-rays; WOLFGANG PAULI considers proteins as colloids; G. BREDIG has a paper on inorganic ferments; RICHARD WILLSTÄTTER writes on adsorption of enzymes; E. B. WILSON discusses physical basis of life; F. D'HERELLE presents an account of the bacteriophage, a living colloidal micell. D. T. MACDOUGAL considers arrangement and action of the colloids of the plant cell; RUDOLPH HÖBER has an account of colloidal state and physiological function; papers by BOTTAZZI, FISCHER, SEIFRIZ, HEILBRUNN, R. S. LILLIE, CHAMBERS, and LUMIÈRE deal with protoplasm as a system of colloids. There are papers on the colloidal features of tuberculosis, malignant tumor, acute inflammation, lobar pneumonia, and cancer.

The appendix reprints a paper by JACQUES LOEB, an explanation of the colloidal behavior of proteins, which was the PASTEUR Lecture delivered before the Institute of Medicine of Chicago, November, 1922. This was in lieu of a special paper which LOEB's untimely death kept him from writing.

The reviewer considers it one of the best collections of papers in this field. The size of the volume makes it expensive, but in these days of high book prices one can easily spend more and obtain less for his money. Libraries should not be without it, if colloidal literature is given a place on the shelves.—C. A. SHULL.

Statistical methods for research workers

The second edition of FISHER'S[4] work on statistical methods, appearing so soon after the first edition (1925), is proof of the popularity of this work, and evidence of the great need for such a monograph for students of biological problems. The problem of variability is always present in any biological analysis, and every man sooner or later finds that he must employ the methods of statistics in testing the accuracy of his results and the validity of his conclusions.

FISHER has tried to avoid the difficulties of the mathematical theory of statistical methods, and aims to present the matter from the standpoint of practical procedure, appropriate to the problems in hand. This permits omission of mathematical proofs, which unfortunately few biologists are well enough trained in mathematics to follow, and yet gives ample opportunity to present

[4] FISHER, R. A., Statistical methods for research workers. 8vo. pp. xi+269. Oliver and Boyd, Edinburgh and London. 1928.

the practical applications of statistics to biological data. FISHER'S wide experience as chief statistician at Rothamsted has brought him into contact with the needs of the man who must cope with small sample problems. He gives us the advantage of his experience in this monograph.

There are nine chapters, the last of which, on principles of statistical estimation, is new in this edition. After an introductory chapter, which considers the scope, methods of calculation, and qualifications of satisfactory statistics, he takes up diagrams; distributions; tests of goodness of fit, independence, and homogeneity; tests of significance of means, differences of means, and regression coefficients; the correlation coefficient; intraclass correlations and the analysis of variance; and further applications of the analysis of variance. The additional ninth chapter takes up the significance of the evidence for linkage, specification of the progeny population for linked factors, the multiplicity of consistent statistics, comparison of statistics by goodness of fit tests, sampling variance of statistics, and the interpretation of the discrepancy χ^2.

A number of the tables most used in actual work are repeated at the end of the book, so that they can be detached and mounted for convenience of reference. These include the table of x, table of x^2, table of t, table of values of the correlation coefficient for different levels of significance, table of r for values of z from 0–3, and tables of the 5 and 1 per cent points of the distribution of z.

The work is not intended to be used as a textbook on statistics, but as a monograph for the research student, to be mastered as a part of his necessary equipment for effective research. Students who lack sufficient mathematics to handle FISHER'S monograph should consider themselves deficient in that fundamental branch of science, and give some attention to algebra and analytics. The book is worthy of careful study by all who deal with problems in which variable data are collected and interpreted.—C. A. SHULL.

NOTES FOR STUDENTS

Taxonomic notes.—HUBBARD[5] has monographed the Australian genus *Astrebla*, which is renowned in that country for its drought resistance and fodder value. It is regarded as one of the "great national assets." Four species are described, one of which is new.

POE[6] has published a revision of the *Plantago patagonica* group, which ASA GRAY first presented as containing one species and 4 varieties. Later studies added to the species, and now POE recognizes 6 species and 9 varieties, the latter being new names or combinations.

SANDWITH[7] has described 14 new species from British Guiana, which have been found in the collections of the last few years. They represent 11 genera and

[5] HUBBARD, C. E., The genus *Astrebla* or Mitchell grasses. Bull. Misc. Inf. Roy. Bot. Gard. Kew, no. 7. 257–266. 1928.

[6] POE, IONE, A revision of the *Plantago patagonica* group of the United States and Canada. Bull. Torr. Bot. Club **55**:406–420. 1928.

[7] SANDWITH, N. Y., New species from British Guiana. Kew Bull. no. 9. pp. 365–379. 1928.

9 families. Attention is especially called to "the discovery of 2 new species of the interesting little genus *Heterostemon*" (Caesalpiniaceae).

In continuation of their descriptions of new plants from tropical Africa, HUTCHINSON and DALZIEL[8] have described 4 new species of *Dichapetalum* (Chailletiaceae), and 8 new species in 5 genera of Caesalpiniaceae.

BARTRAM[9] has published descriptions of the mosses collected by STANDLEY in Costa Rica, which he visited in the early part of 1924 and the winter of 1925–26 to investigate the flowering plants. He enumerates 272 species and varieties, representing 40 families, over one-third of which are new to Costa Rica. He also describes 42 new species and varieties, and a new genus (*Neophypnella*) belonging to the Hookeriaceae. This indicates the wealth of moss material in a region of Central America where mosses have not been collected. It is stated that "the wet mountain forests of Central America are rich in mosses, especially in epiphytic forms."

FERNALD[10] has published an account of the species of *Oxytropis* occurring in the northeastern section of North America. It is so prominently a genus of western North America that he remarks that "its occurrence in the northeastern section of North America has been looked upon as exceptional." He describes and discusses 10 species, which include one new species and 2 new combinations. —J. M. C.

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[8] HUTCHINSON, J., and DALZIEL, J. M., Tropical African plants. V. Kew Bull. no. 9. pp. 380–382. 1928.

[9] BARTRAM, E. B., Costa Rican mosses collected by PAUL C. STANDLEY in 1924–1926. Contrib. U.S. Nat. Herb. **26**: Part 3. 51–114. 1928.

[10] FERNALD, M. L., The genus *Oxytropis* in northeastern America. Contrib. Gray Herb. LXXXII. Reprinted from Rhodora **30**:137–188. 1928.

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